



LIBRARY
New Delhi

Call No. _____

Acc. No. 14628

Indian Agricultural Research Institute (Pusa)
LIBRARY, NEW DELHI-110012

This book can be issued on or after _____

Return Date	Return Date

CEREAL CHEMISTRY

Published by the American Association of Cereal Chemists

Editor-in-Chief - - - M. J. BLISH
Assistant Editor - - - R. T. PRESCOTT
Managing Editor - - - R. M. SANDSTEDT

Associate Editors

O. E. STAMBERG	CLINTON L. BROOKE
C. O. SWANSON	W. F. GEDDES



VOLUME XIX—Nos. 1-6
JANUARY-NOVEMBER, 1942



Lancaster, Pennsylvania

1942

mined by the photometric method. Bran contains about 17.5% protein, consisting of globulin and albumin in addition to considerable quantities of a prolamine and probably a glutelin. It might be expected that the latter two proteins could not be differentiated photometrically from the gluten proteins, but tests on relatively pure bran indicate that these proteins produce considerably less turbidity under the conditions of the test than do the corresponding proteins (the gluten proteins) of the endosperm. It may be stated, then, that the photometric method does differentiate in a large measure between the gluten and the nongluten proteins of the wheat and that the test is nearly, although not entirely, specific for the gluten proteins.

The Method

The photometric method used in this study was found to be applicable to both wheat and wheat flour. The details of the procedure are given below. This method differs in certain minor respects from the one previously reported for wheat flour (see footnote 4).

1. To exactly 0.5 g of the freshly and finely ground, well-mixed sample in a 130-ml centrifuge tube that can be stoppered, add 100 ml of 0.05*N* KOH solution.

2. Shake the stoppered tube intermittently for about 3 minutes, remove the stopper, and centrifuge for 10 minutes at approximately 1800 rpm. (In the case of flour lumps may form which must be completely broken up during the shaking process before centrifuging.)

3. To exactly 5 ml of the centrifugate in a photometer test tube (one of the selected tubes for use in lieu of an absorption cell), add exactly 25 ml of a buffer solution made by mixing 6 parts by volume of 0.2*M* KH_2PO_4 with 94 parts by volume of 0.2*M* Na_2HPO_4 . This buffer solution should have a pH of 7.8 and should be preserved by the addition of 1 ml of toluene per liter of solution. Mix the contents of the test tube by inversion and allow to stand for 1 hour.

4. Determine the transmission of light through the solution in the test tube with a photoelectric photometer, using a light filter having a maximum transmission at a wave length of 530 millimicrons. (Other wave lengths will give different but equally satisfactory results.)

Determination of Endosperm Protein

Since the readings obtained by the photometer presumably are dependent upon the gluten protein rather than upon the total protein content of the sample and since the ratio of gluten protein to total protein varies widely among different samples of wheat, an accurate evaluation of the reliability of the photometric results as a measure of gluten protein can be made only by a direct comparison of photometric readings with gluten content values.

Unfortunately, however, there appears to be no direct and accurate method for determining the gluten protein content of wheat other than the extremely tedious and impractical procedure of dissecting the pure endosperm from a large number of kernels and carrying out peptization studies on the dissected material. In the absence of a practical direct method we have endeavored to calculate the endosperm protein content of wheat indirectly from the total protein and ash content of the sample. Although pure endosperm probably contains proteins other than the gluten proteins it is assumed that the content of such non-gluten protein in the endosperm is very small.

If the ash content of endosperm and the nonendosperm portion of a sample of wheat were known, the percentages of endosperm and non-endosperm constituents could be calculated by simple proportion using the equations:

$$N = \frac{100(A_W - A_E)}{A_N - A_E} \quad (1)$$

$$E = 100 - N \quad (2)$$

where N = % nonendosperm, E = % endosperm, A_W = % ash in wheat, A_E = % ash in endosperm, and A_N = % ash in nonendosperm.

Since pure bran and pure germ contain close to 9% and 5% of ash respectively and since the ratio of bran to germ in the wheat kernel is ordinarily about 13 : 2.5, the ash content of the nonendosperm portion of the wheat kernel is calculated to be approximately 8.4%. If it is assumed that the ash content of pure wheat endosperm is 0.35%, equation No. 1 may be simplified to:

$$N = \frac{100(A_W - 0.35)}{8.05} \quad (3)$$

Although the values for ash content of the endosperm and nonendosperm may vary from those used in equation No. 3, the difference between the two values is so great that the normal variability of either of them will have but little effect on the calculated value of N .

In the general run of hard red winter wheat the ratio of bran weight to germ weight may be considered to be reasonably constant, the weight of endosperm being the most variable factor. The range in wheat ash ordinarily encountered corresponds to a range in the non-endosperm constituents of from 10% to 30% as calculated from equation No. 3, and the range in germ content is generally considered to be from 2% to 3% of the wheat. Using these values, the germ content and the bran content of any sample may be calculated from the non-endosperm content by means of the following equations:

$$G = 0.05N + 1.5 \quad (4)$$

and

$$B = N - G \quad (5)$$

where G = % germ, B = % bran, and N = % nonendosperm (from equation No. 3).

Since pure bran is said to contain approximately 17.5% protein and pure germ 40% protein, the bran and germ protein contents of the original wheat may be determined by using the values obtained in equations No. 4 and No. 5. The endosperm protein is then obtained by subtracting the sum of the bran and germ protein from the total protein value.

The above method for determining the endosperm protein content of wheat is based on the assumption that the variation in ash content among different samples of wheat is due to the variation in the proportions of the three major structures of the wheat kernel, endosperm, germ, and bran, and not to a variation in the ash content of the individual structures themselves. It is also based on the assumption that variation in wheat protein is due primarily to variation in relative size and protein content of the endosperm, and that the protein content of pure bran and of pure germ is relatively constant. Obviously these assumptions are not strictly true and the calculated values for endosperm protein cannot, therefore, be considered highly accurate. Except in unusual instances, however, it is not likely that variabilities in the values assumed to be constant are sufficiently great to cause serious errors in this method of calculation.

Experimental Work

One hundred and ninety-five samples of commercial hard red winter wheat of the 1940 crop from 15 different states were analyzed by the photometric method and the light transmission values compared with the protein content values determined by the Kjeldahl method. The results are listed in Table I and are shown graphically in Figure I.

From a study of the graphical presentation of these data (Fig. 1) it became evident that in the case of wheats that were characterized by low test weight, high ash content, and a shriveled appearance, light transmission values usually indicated lower protein contents than those shown by Kjeldahl analysis. Such wheats contain more bran and less endosperm than normal wheats and consequently show a lower than average ratio of gluten protein to total protein. Conversely in the case of wheats that were characterized by high test weight, low ash content, and a plump appearance, light transmission values usually indicated higher protein contents than those shown by Kjeldahl analysis. Wheats of this type contain less bran and more endosperm than average wheats and hence show a higher than average ratio of gluten protein to total protein. These observations support the theory

TABLE I

TOTAL PROTEIN, ASH, ENDOSPERM PROTEIN (CALCULATED), AND LIGHT TRANSMISSION VALUES ON 195 SAMPLES OF HARD RED WINTER WHEAT FROM THE 1940 CROP

(Samples are listed in order of increasing total protein content. All data are on an "as is" moisture basis.)

Sample No.	State	Total protein (Kjeldahl)	Ash	Endosperm protein	Light transmission
		%	%	%	%
137	Idaho	8.23	1.47	5.30	76.5
455	Washington	8.28	1.62	5.00	75.2
439	Washington	8.38	1.35	5.72	72.2
621	Washington	8.53	1.62	5.25	73.4
540	Montana	8.62	1.76	5.00	76.3
1000	Indiana	8.66	1.82	4.92	73.0
614	Washington	8.73	1.48	5.77	70.3
139	Idaho	8.80	1.72	5.27	72.9
145	Washington	8.96	1.68	5.54	73.2
147	Idaho	9.04	1.54	5.95	73.0
148	Washington	9.05	1.62	5.77	72.6
858	Montana	9.06	1.77	5.42	73.5
479	Washington	9.10	1.68	5.86	71.8
433	Washington	9.33	1.46	6.41	69.9
480	Idaho	9.38	1.78	5.72	69.5
489	Idaho	9.38	1.76	5.76	69.5
163	Washington	9.43	1.58	6.22	69.3
451	Montana	9.48	1.68	6.06	70.3
486	Washington	9.50	1.73	5.95	69.6
475	Washington	9.52	1.62	6.24	68.7
985	Indiana	9.53	1.75	5.94	69.8
806	Kansas	9.53	1.74	5.96	71.5
143	Washington	9.53	1.61	6.28	69.9
443	Washington	9.55	1.70	6.09	69.3
173	Washington	9.64	1.60	6.39	68.2
155	Washington	9.64	1.39	6.91	67.9
628	Washington	9.68	1.65	6.33	71.3
456	Washington	9.68	1.58	6.47	71.1
135	Washington	9.70	1.61	6.45	70.5
171	Washington	9.71	1.62	6.43	69.8
437	Washington	9.75	1.47	6.82	70.5
129	Washington	9.76	1.79	6.09	72.6
141	Washington	9.77	1.64	6.45	69.5
612	Washington	9.78	1.49	6.80	68.2
854	Montana	9.79	1.70	6.33	69.8
374	Illinois	9.79	1.68	6.37	70.1
631	Washington	9.81	1.48	6.85	68.6
467	Washington	9.86	1.58	6.65	70.2
385	Illinois	9.87	1.77	6.23	69.4
453	Washington	9.92	1.42	7.10	68.1
436	Washington	9.93	1.59	6.72	66.7
617	Washington	9.93	1.46	7.01	67.1
472	Washington	9.96	1.45	7.07	69.3
174	Idaho	9.97	1.79	6.30	70.0
451	Washington	10.04	1.35	7.38	67.2
447	Oregon	10.05	1.95	6.00	70.0
474	Washington	10.07	1.59	6.86	70.6
756	Kansas	10.11	1.56	6.99	68.5
694	Illinois	10.25	1.89	6.33	71.3
718	Illinois	10.35	1.93	6.34	69.9
478	Washington	10.42	1.50	7.42	65.9
672	Illinois	10.46	1.71	6.98	69.4

TABLE I—*Continued*

Sample No.	State	Total protein (Kjeldahl)	Ash	Endosperm protein	Light transmission
		%	%	%	%
857	Minnesota	10.57	2.08	6.20	66.5
998	Indiana	10.62	1.87	6.77	67.9
562	Kansas	10.62	1.77	6.98	68.3
997	Indiana	10.67	1.84	6.87	68.9
673	Illinois	10.70	1.81	6.97	68.5
737	Kansas	10.89	1.49	7.91	64.5
675	Illinois	11.10	1.84	7.30	66.8
715	Illinois	11.22	1.87	7.37	68.2
671	Illinois	11.23	1.74	7.66	65.5
595	Kansas	11.27	1.78	7.61	64.8
609	Kansas	11.32	1.76	7.70	63.2
681	Illinois	11.32	1.71	7.84	68.4
683	Missouri	11.38	1.87	7.53	66.7
674	Illinois	11.41	1.81	7.68	66.9
682	Illinois	11.46	1.83	7.70	65.6
984	Illinois	11.47	1.81	7.74	64.2
600	Kansas	11.52	2.02	7.32	64.5
865	Iowa	11.52	1.86	7.69	68.7
844	Montana	11.56	1.67	8.15	62.9
680	Illinois	11.58	1.77	7.94	65.0
869	Iowa	11.59	1.88	7.72	64.4
702	Kansas	11.60	1.66	8.23	62.3
729	Kansas	11.61	1.59	8.40	63.1
676	Oklahoma	11.72	1.78	8.06	63.8
748	Oklahoma	11.77	1.85	7.96	65.2
866	Iowa	11.80	2.05	7.54	64.4
592	Kansas	11.80	1.65	8.45	61.5
678	Illinois	11.83	1.78	8.17	63.9
1001	Iowa	11.91	1.62	8.63	62.2
571	Nebraska	11.92	1.74	8.35	63.8
632	Oregon	12.03	1.77	8.39	63.8
584	Kansas	12.04	1.66	8.67	60.8
604	Kansas	12.04	1.57	8.86	61.4
665	Oregon	12.07	1.76	8.45	62.9
579	Kansas	12.12	1.77	8.48	63.6
961	Oklahoma	12.12	1.78	8.46	61.8
846	Montana	12.21	1.57	9.03	62.3
606	Kansas	12.28	1.88	8.41	60.0
599	Kansas	12.30	1.63	9.00	59.9
589	Nebraska	12.40	1.81	8.67	61.2
711	Kansas	12.42	1.84	8.62	60.8
835	Iowa	12.47	1.76	8.85	61.4
567	Kansas	12.48	1.70	9.02	61.0
1086	Texas	12.54	1.73	8.99	62.5
789	Iowa	12.71	1.89	8.79	62.2
561	Kansas	12.75	1.78	9.09	59.9
629	Montana	12.76	1.58	9.55	61.0
825	Iowa	12.81	1.92	8.82	61.6
713	Oklahoma	12.86	1.76	9.24	59.2
588	Oklahoma	12.87	1.80	9.18	59.0
620	Montana	12.91	1.62	9.63	59.2
828	Iowa	12.92	1.96	8.86	61.7
568	Kansas	13.26	1.63	9.96	57.4
586	Kansas	13.37	1.78	9.71	57.9
861	Iowa	13.49	1.77	9.85	58.3
952	Oklahoma	13.62	1.69	10.18	55.1

TABLE I—Continued

Sample No.	State	Total protein (Kjeldahl), %	Asi, %	Endosperm protein, %	Light transmission, %
1031	Iowa	13.62	1.71	10.14	55.9
686	Oklahoma	13.68	1.61	10.43	56.0
692	Oklahoma	13.73	1.88	9.86	56.8
630	Montana	13.76	1.49	10.78	56.6
940	Oklahoma	13.87	1.74	10.30	57.8
573	Kansas	13.89	1.81	10.16	58.4
685	Oklahoma	13.97	1.82	10.23	54.4
615	Montana	14.09	1.46	11.17	53.7
623	Montana	14.11	1.50	11.11	56.7
618	Montana	14.16	1.56	11.04	53.8
613	Montana	14.26	1.39	11.53	53.4
785	Nebraska	14.32	1.76	10.70	55.5
684	Oklahoma	14.37	1.92	10.38	56.9
873	Wyoming	14.37	1.70	10.91	56.7
622	Montana	14.47	1.67	11.06	53.9
791	Nebraska	14.51	1.84	10.71	56.7
605	Kansas	14.51	1.73	10.96	52.5
783	Nebraska	14.56	1.88	10.69	56.9
788	Nebraska	14.56	1.77	10.92	52.6
601	Kansas	14.56	1.65	11.21	55.7
624	Montana	14.69	1.58	11.48	52.2
627	Montana	14.71	1.59	11.59	57.2
790	Nebraska	14.78	1.75	11.19	55.3
559	Nebraska	14.80	1.78	11.14	53.9
578	Kansas	14.87	1.89	10.95	54.3
616	Montana	14.88	1.35	12.22	49.2
784	Nebraska	14.92	1.92	10.93	56.2
843	Montana	14.96	1.66	11.59	51.7
572	Kansas	15.06	1.76	11.44	56.4
574	Oklahoma	15.12	1.76	11.55	51.3
677	Oklahoma	15.13	1.89	11.21	52.2
564	Nebraska	15.20	1.77	11.56	53.3
747	Texas	15.21	1.86	11.38	52.1
786	Nebraska	15.34	1.79	11.67	52.2
745	Texas	15.35	2.02	11.15	52.6
679	Texas	15.36	1.99	11.23	50.2
587	Kansas	15.56	2.06	11.25	54.6
602	Nebraska	15.56	1.98	11.44	53.3
590	Kansas	15.58	1.83	11.82	50.4
576	Kansas	15.66	1.65	12.31	50.8
619	Montana	15.66	1.47	12.73	50.7
734	Oklahoma	15.69	1.73	12.14	52.3
591	Nebraska	15.71	1.70	12.25	49.0
625	Montana	15.74	1.59	12.53	49.9
913	Wyoming	15.6	1.69	12.32	50.7
585	Kansas	16.09	1.86	12.26	50.6
607	Kansas	16.31	2.17	11.77	51.2
691	Oklahoma	16.38	1.71	12.90	48.6
603	Kansas	16.41	2.01	12.22	50.3
693	Oklahoma	16.51	1.86	12.68	49.1
626	Montana	16.51	1.61	13.26	51.5
592	Kansas	16.54	2.19	11.95	52.6
593	Nebraska	16.63	2.04	12.39	49.4
575	Kansas	16.76	2.25	12.02	49.9
586	Nebraska	16.81	2.19	12.22	51.0

TABLE I—*Continued*

Sample No	State	Total protein (Kjeldahl)	Ash	Endosperm protein	Light transmission
		%	%	%	%
849	Colorado	16.83	1.94	12.80	48.7
594	Kansas	16.84	1.83	13.08	48.9
610	Montana	16.91	1.61	13.66	48.7
577	Kansas	16.94	2.10	12.54	48.3
787	Nebraska	16.99	1.94	12.96	48.8
1085	Texas	17.00	1.88	13.13	46.9
896	Wyoming	17.16	1.89	13.24	50.3
1078	Texas	17.36	2.03	13.14	45.5
797	Kansas	17.39	2.22	12.70	47.7
583	Kansas	17.40	2.14	12.91	50.1
581	Nebraska	17.41	2.06	13.10	48.5
565	Kansas	17.47	2.03	13.25	48.0
569	Kansas	17.68	2.21	13.03	46.1
560	Nebraska	17.74	2.18	13.18	49.5
608	Nebraska	17.91	2.24	13.19	45.7
598	Kansas	17.98	2.25	13.24	48.1
570	Kansas	17.99	2.18	13.43	48.5
527	Colorado	18.03	2.17	13.49	45.8
566	Nebraska	18.04	2.03	13.82	45.9
543	Kansas	18.15	2.33	13.25	47.6
596	Kansas	18.19	2.08	13.82	45.2
518	Kansas	18.22	2.29	13.39	46.5
563	Kansas	18.32	2.49	13.03	47.8
597	Kansas	18.32	2.25	13.58	47.4
58	Kansas	18.36	2.34	13.45	46.1
28	Kansas	18.45	2.32	13.57	43.3
23	Kansas	18.48	2.15	13.97	44.2
72	Kansas	18.65	2.33	13.75	43.6
11	Kansas	18.65	2.32	13.77	43.3
84	Kansas	18.71	2.26	13.94	45.2
40	Kansas	18.76	2.54	13.39	47.1
59	Kansas	18.82	2.13	14.35	44.5

that light transmission values are a better index of gluten protein than of total protein content.

Approximate values of endosperm protein content for the series of samples under investigation were determined by the method herein described. These values also are listed in Table I and are shown graphically in Figure 2. A comparison of Figures 1 and 2 indicates that the endosperm protein appears to bear a linear relationship to the light transmission but that the relationship between total protein and light transmission is curvilinear. For the purposes of statistical analysis it was found that this latter relationship could be made linear by using the arcsine of the total protein content rather than the total protein content itself.⁵

The following statistical values for correlation coefficient and standard errors of estimate were obtained:

⁵For a discussion of this technique see G. W. Snedecor, Statistical methods (3rd ed.), p. 382 (1940), the Iowa State College Press.

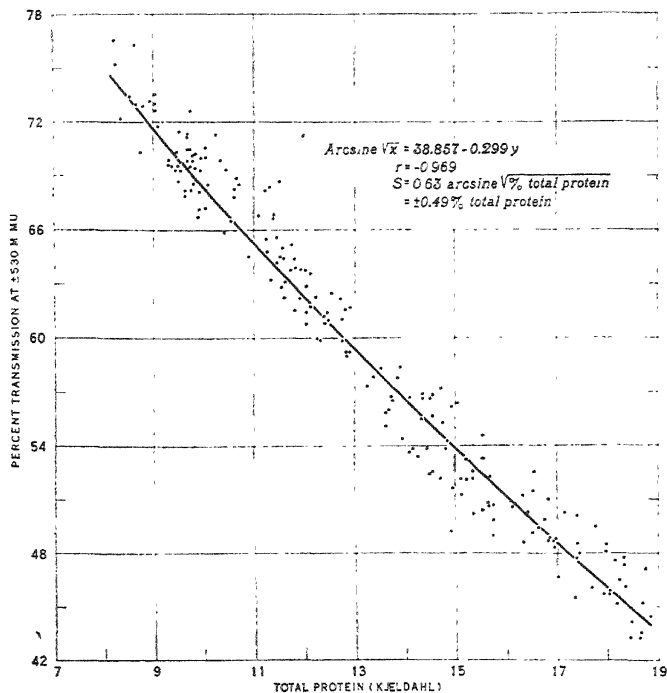


Fig. 1. Total protein and light transmission values on 195 samples of hard red winter wheat.

For total protein and light transmission

$$r = -0.969$$

$S = 0.634$ arcsine of the total protein percentage or approximately 0.49% total protein.

For endosperm protein and light transmission

$$r = -0.987$$

$$S = 0.44 \text{ } \sqrt{\% \text{ endosperm protein}}$$

The further observation was made that in the case of the endosperm protein data (Fig. 2) 76% of the values fell within 0.5% (in terms of endosperm protein) of the regression line, while in the case of the total protein data (Fig. 1), only 62% of the values fell within 0.5% of the theoretical curve representing the relationship between total light transmission and total protein. Thus the light transmission values are shown to be a somewhat better measure of endosperm protein (and therefore presumably of gluten protein) than of total protein content. This observation lends further support to our theoretical contention.

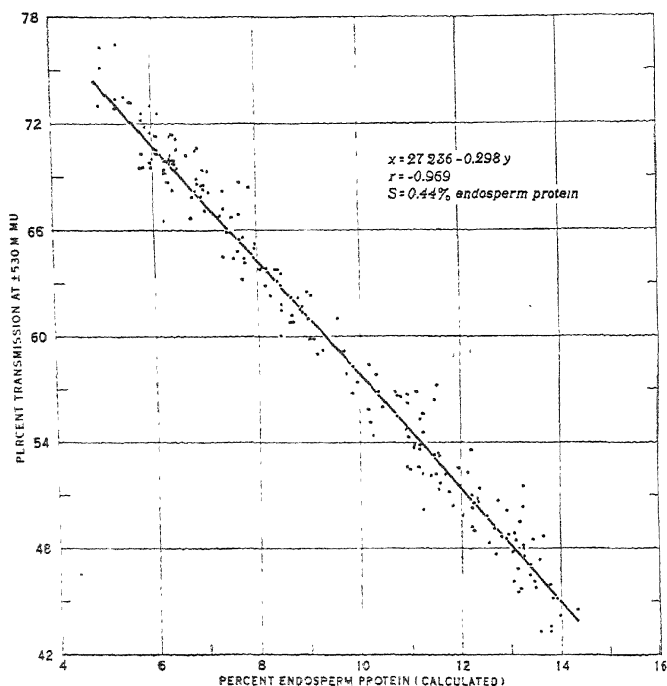


Fig. 2—Endosperm protein 'calculated' and light transmission values on 195 samples of hard red winter wheat.

Summary

The photometric method previously reported for the determination of protein in wheat flour has been applied, with minor modifications, to the analysis of wheat. Theoretically the method differentiates to a considerable degree between gluten and nongluten protein and thus comes closer to being a measure of gluten protein than of total protein. Experimental evidence substantiating this theory is presented.

The principal advantages of the photometric method for routine protein analysis should be the ease and rapidity with which a large volume of work can be handled with relatively simple equipment and without the unpleasant features usually associated with a protein laboratory. An additional advantage is that the results obtained are probably a somewhat better index of ultimate baking quality than are the values obtained by the conventional Kjeldahl procedure.

Further work will be directed toward the adequate standardization of the method and of the photometers used in order that concordant results may be obtained among different laboratories.

Acknowledgment

The assistance of H. C. Fellows and Louis Feinstein of the Agricultural Marketing Service, who made the Kjeldahl protein determinations, and of O. E. Rauchschalbe of the Bureau of Plant Industry, who conducted the statistical analysis of the data, is gratefully acknowledged.

THE INFLUENCE OF TEMPERATURE ON THE DEVELOPMENT OF AMYLASE IN GERMINATING WHEAT¹

ERIC KNEEN, BYRON S. MILLER, and R. M. SANDSTEDT

Departments of Agricultural Chemistry and Plant Pathology, Nebraska Agricultural Experiment Station, Lincoln, Nebraska

(Read at the Annual Meeting, May 1941)

The importance of amylolytic action in the brewing, distilling, and other industries and in the supplementation of bread flours has stimulated much investigation of the development of amylase during the germination of cereal grains. Early studies were mainly evaluations of the increase in saccharogenic power during the production of malt. The work of Lintner (1887) and Brown and Morris (1890) did much to clarify the conception of amylase development during malting. These authors found that malt diastatic power was the result of the action of two components: a sugar-producing enzyme similar to that in the original ungerminated grain and a "liquefying" enzyme appearing during germination. Through the following years the "two enzyme" theory developed but it was not until the work of Ohlsson (1926, 1930) that a means was provided for following the development of the individual amylase components, alpha- (dextrinogenic) and beta- (saccharogenic) amylase, during germination of grain. Ohlsson and co-workers (Nordh and Ohlsson, 1932; Ohlsson and Uddenberg, 1933; Ohlsson and Edfeldt, 1933; Stenstam, Björling, and Ohlsson, 1934; Ohlsson and Thörn, 1938), Lüers and Rümmler (1935), and Ugrumow (1935) used this differential inactivation technique (70°C to inactivate beta-amylase, pH 3.3 to inactivate alpha-amylase) to study the elaboration of these enzymes during germination of barley, wheat, rye, and oats. Their researches indicate that beta-amylase activity increases in the early stages of seedling development and attains a maximum after a few days, and that alpha-amylase activity appears

¹Published with the approval of the Director as paper No. 290, Journal Series, Nebraska Agricultural Experiment Station.

after a day or so of germination and increases throughout the germination period.

Following a somewhat different approach, many investigations have been made on the nature of the increase in saccharogenic power during germination. The work of Ford and Guthrie (1908a, b) demonstrated that the action of proteolytic enzymes liberated additional saccharogenic amylase from ungerminated barley and wheat. The researches of several authors, notably Weichherz and Asmus (1931), Waldschmidt-Leitz and Purr (1931), Myrbäck and Myrbäck (1933), Lüers and Lechner (1933), Chrzaszcz and Janicki (1933) and Lüers and Rümmler (1933), resulted in the conclusion that the increase in saccharifying activity coincident with germination is largely the result of the liberation of the "bound" amylase of the ungerminated cereal by the action of the proteolytic enzymes simultaneously developing during germination. More recent work of Hills and Bailey (1938a, b) and of Snider (1941) indicates that malt saccharogenic activity is dependent upon the combined action of the beta-amylase, that originally present in the "free" state in grain plus that liberated by proteolytic action during malting, and the alpha-amylase formed or activated in the germination process.

The literature² provides no data relating exclusively to the development of the *individual* amylase components during germination. The importance of considering the activities of these individual components in malt studies has recently been emphasized by Kneen, Beckord, and Sandstedt (1941). Further, Kneen and Sandstedt (1941b) clearly demonstrate that the value of malt as a flour supplement is dependent upon the content of only one component, alpha-amylase. It is important then to re-examine the nature of the development of the amylases during the germination of cereal grains.

The Ohlsson (1930) technique of differential inactivation of the amylases is at best only qualitative. The heat treatment results in the loss of a portion of the alpha component, while the acid treatment not only inactivates a part of the beta component but rarely effects complete inactivation of alpha-amylase. Recent methods of Sandstedt, Kneen, and Blish (1939) and Kneen and Sandstedt (1941a) have made practical the determination of these individual components without such differential inactivation. The purpose of the present study was to use these methods to measure accurately the development of the amylase components in germinating wheat as influenced by germination temperature.

² Additional literature review may be found in the articles by Lüers (1936) and Weichherz and Asmus (1931).

Methods

Preparation of wheat: A sample of hard red winter wheat was carefully sorted and all broken or badly shriveled kernels removed. Twelve samples having 500 seeds each were counted out and weighed. The average weight per 1,000 kernels was found to be 20.06 g. Samples used for germination were 30 g each or approximately 1,500 seeds. No seed treatment other than the 12-hour pregermination soak was used.

Germination: The 30-g samples of wheat were soaked in distilled water for 12 hours at room temperature and then transferred to "rag dolls"³ for germination. The dolls were immediately placed in germination cabinets at the desired temperature. Each doll contained a 30-g sample of wheat and a sufficient number were maintained at each temperature so that the contents of a whole doll were available for each analysis. Four cabinets were used, each being held at constant temperature throughout the experiment. The germination temperatures used were 20°, 15°, 10°, and 5°C. Humidity was kept at a high level in all cabinets. This method of germination proved particularly desirable for enzyme studies during germination of seeds not previously given a fungicidal treatment. Some mold growth did appear towards the last of the germination period in the dolls held at 20°C and at 15°C, but none was apparent at any time at 10° or 5°C. The few moldy seedlings occurring were removed after the samples were weighed and dried.

Sampling: At the end of the desired period of germination (measured from the time the dolls were placed in the germinator) the contents of a rag doll were removed quantitatively to a moisture can and total green weight determined. Immediately following the weighing, a sample was taken for the determination of dry-matter content. The remainder was spread out thinly under high-speed fans and dried at room temperature. An estimate of sprout length was made at this time. When the sample was dry it was ground finely in a burr mill and samples taken for dry-matter determination and for extraction.

Extraction: Two types of extracts were prepared, (1) a "free" extract resulting from a 3-hour extraction at 30°C of 5 g of the meal with 50 ml of dilute calcium acetate solution (1 mg calcium acetate per 1 ml) and (2) a total extract resulting from an 18-hour extraction at 30°C of 5 g of meal plus 0.2 g of Merck's papain with 50 ml of the dilute calcium acetate solution. These extracts were diluted to concentrations adapted to the determinations of saccharifying and alpha-dextrinizing activities. It was realized that the choice of a 3-hour

³ The modified rag doll commonly used in agronomic practice for germinating corn is described by Duddleston (1920).

period for the "free" and an 18-hour period for the papain extraction was arbitrary. These times roughly correspond to those used by other workers. It is doubtful if *any* water extract of malt can properly be termed a free extract, since liberation of the "bound" amylase by the proteolytic enzymes present in the germinated grain itself is initiated as soon as extraction commences. However, these techniques do give some idea as to the relative availability of the amylases in different samples. The papain used was of adequate activity; greater quantities or activation treatments resulted in no greater extraction of the amylases.

Determination of amylase activities: Saccharogenic activity and beta-saccharogenic activity were determined according to the technique of Kneen and Sandstedt (1941a) and are reported as saccharogenic and beta-amylase units respectively. Alpha-dextrinogenic activity on those samples having more than two units of alpha-amylase activity was determined by the method of Sandstedt, Kneen, and Blish (1939). With those samples having less than two units of activity the method of Sandstedt and Kneen (1941) for the determination of small quantities of alpha-amylase was used. This "micro method" for alpha-amylase consists essentially of allowing the extract to act on the buffered alpha-amylodextrin solution at 30°C for some 18 hours. At the end of this period a known amount of alpha-amylase is added and the time taken to reach the standard end point with iodine is determined. The difference between this dextrinization time and that for the known amount of alpha-amylase is a measure of the degree of dextrinization attributable to the small quantity of alpha-amylase present in the unknown extract. As in the "macro method" alpha-amylase activity is expressed as "the number of grams of soluble starch which, under the influence of an excess of beta-amylase, are dextrinized by one gram of malt in one hour at 30°C."

Extracts equivalent to 0.01 g of malt were found satisfactory for all the determinations of saccharogenic activity. As seedling growth progressed it was found advisable to vary the weights used for the determination of alpha-amylase activity. In the "micro method" these weight equivalents varied from 0.2 g of the ungerminated grain to 0.01 g of a malt having approximately two units of alpha-amylase activity. In the "macro method" weight equivalents ranged from 0.2 to 0.01 g of malt.

Results

All the data pertinent to the germination study are given in Table I. This table is divided into four sections to cover the results of germination at the four temperatures, 20°, 15°, 10°, and 5°C. For each germination period data are presented for total green and total dry weight

TABLE I
WEIGHT CHANGES, SPROUT ELONGATION, AND AMYLASE DEVELOPMENT OF
WHEAT GERMINATING AT THE FOUR TEMPERATURES OF
20°, 15°, 10°, AND 5°C

Days germi- nated	Total green weight	Total dry weight	Sprout length	Saccharogenic activity		Beta-amylase activity		Alpha-amylase activity	
				"Free"	Total	"Free"	Total	"Free"	Total
	g	g	mm	units	units	units	units	units	units
GERMINATION TEMPERATURE: 20°C									
0	30.0	26.7	—	10.1	28.4	10.1	28.4	0.027	0.043
½	47.7	27.8	—	11.0	28.6	11.0	28.6	0.351	0.359
1	49.1	27.2	1-2	12.8	29.3	12.7	29.2	1.83	1.95
1½	50.4	27.0	2-3	14.2	28.6	13.8	28.1	8.50	9.40
2	53.5	27.1	3-4	17.3	28.5	16.1	27.2	24.0	23.8
2½	57.1	27.2	5-7	22.2	31.4	19.3	28.5	55.6	56.9
3	55.1	25.1	6-9	25.2	32.0	21.5	28.1	72.8	79.4
3½	62.4	21.8	10-20	30.1	35.3	23.3	27.6	138	153
4	64.7	19.0	20-30	37.7	39.1	26.0	27.2	238	242
GERMINATION TEMPERATURE: 15°C									
0	30.0	26.7	—	10.1	28.4	10.1	28.4	0.027	0.043
½	47.2	27.7	—	10.7	29.3	10.7	29.3	0.097	0.122
1	48.1	27.2	0-1	11.9	29.3	11.9	29.3	0.595	0.546
1½	49.2	27.3	2-2	11.8	28.2	11.7	28.1	1.87	2.34
2	50.7	26.9	1-2	13.3	28.3	13.0	28.0	6.00	6.37
2½	52.2	27.4	2-3	16.9	31.6	16.0	30.7	18.1	18.7
3	54.7	27.1	3-5	18.1	30.6	16.3	28.6	35.0	40.5
4	61.8	27.0	5-10	25.1	31.1	20.8	26.5	87.2	90.7
5	78.8	25.8	20-25	37.9	37.9	26.6	26.2	229	238
6	89.5	26.5	20-30	39.1	39.0	26.9	26.3	250	260
GERMINATION TEMPERATURE: 10°C									
0	30.0	26.7	—	10.1	28.4	10.1	28.4	0.027	0.043
1	47.6	27.3	—	11.6	29.8	11.6	29.8	0.170	0.120
2	49.3	27.3	—	12.1	30.9	12.1	30.9	0.918	1.02
3	51.6	27.0	1-2	13.0	30.7	12.7	30.4	5.22	5.68
4	53.1	26.8	2-4	17.3	30.4	16.0	28.8	26.6	31.2
5	55.8	26.9	3-5	20.1	31.9	17.7	29.3	47.6	51.3
6	60.0	27.2	5-6	24.4	34.4	20.3	30.2	80.6	83.2
7	65.7	26.1	7-10	28.0	36.4	22.3	29.5	114	134
8	73.9	26.1	10-14	30.9	37.3	23.4	28.8	148	167
9	88.1	25.3	15-25	35.0	38.0	24.9	27.3	205	217
10	99.2	25.7	25-35	41.1	40.7	28.2	27.2	265	281
GERMINATION TEMPERATURE: 5°C									
0	30.0	26.7	—	10.1	28.4	10.1	28.4	0.027	0.043
1	47.7	28.4	—	10.9	28.3	10.9	28.3	0.080	0.082
2	48.1	27.1	—	11.8	29.4	11.8	29.4	0.222	0.375
3	49.3	26.9	0-1	9.5	29.3	9.5	29.3	0.780	0.800
4	49.8	26.8	1-1	8.9	29.5	8.9	29.4	1.20	1.72
5	50.6	27.2	1-1½	9.3	30.4	9.2	30.2	2.34	4.07
6	50.7	27.0	1-2	12.9	30.1	12.6	29.8	6.11	6.24
8	52.5	26.7	1-3	13.2	32.3	12.5	31.5	14.1	15.8
10	53.5	26.8	1-3	14.0	31.1	12.9	29.7	22.2	26.8
12	55.4	26.4	2-3	17.9	31.1	16.0	28.7	37.9	48.5
16	60.9	26.1	4-7	23.0	34.4	18.5	29.6	92.2	94.5
20	74.4	25.9	12-17	34.0	37.9	25.2	28.8	177	182
24	95.5	22.7	25-35	40.3	40.8	27.9	27.9	258	265

of the sample, for approximate sprout length, and for the various amylolytic activities of both the "free" and the total (papain) extracts. Saccharogenic and beta-amylase units are those of Kneen and Sandstedt (1941a) and alpha-amylase units those of Sandstedt, Kneen, and Blish (1939). In all instances then the amylase activity is referred to as the starch hydrolysis caused by one gram of the dry meal.

Germination at each temperature was terminated when sprout length reached 30 to 35 mm. For adequate data it was necessary to sample every 12 hours at the 20° germination temperature and at least daily at 15° and 10°C. On the other hand germination progressed slowly at 5° and sampling was not done so frequently. It should be re-emphasized that a germination period of one day indicates one day in the rag doll *after* soaking and that the data of Table I refer to the whole seedling.

Seedling weight and sprout length: Gaumann (1932), in extensive research on the germination of wheat, found that velocity of growth was more rapid and loss of dry weight more pronounced at high temperatures than at low. The data of Table I support these findings. At 20°, 15°, 10°, and 5°C, the times necessary to produce sprouts of from 30 to 35 mm length were respectively 4, 6, 10, and 24 days. At the end of the germination periods the green weights of the 15°, 10°, and 5° samples were triple that of the original grain. However, at 20° the original weight was only doubled. Very little loss of dry weight occurred at either 15° or 10°C. Some loss was apparent during the long period at 5°C and a very decided loss during the four days at 20°C. This rapid loss in dry weight at high temperatures of germination, combined with the tendency of the sample to dry out and to acquire mold growth, made germination at 20°C a rather unsatisfactory procedure.

Influence of pregermination treatment: Ohlsson and co-workers (1932-1938) included the period of soaking (as long as 3 days) in the germination period. Stenstam, Björling, and Ohlsson (1934) reported that, in germinating wheat, the extractable beta-amylase decreased during this soaking treatment. Their macro method for alpha-amylase indicated an absence of this component during the preliminary treatment. To investigate any possible changes taking place during the pregermination treatment, samples soaked for 12 and 24 hours were included in the analysis. Table II shows the results obtained. Data for the untreated wheat sample and for 12 hours of germination at 15°C are included for comparison. The data indicate that changes in extractable beta-amylase, either free or total, are insignificant during soaking. However, this treatment does seem to result in some increase in both free and total alpha-amylase, particularly during the first 12 hours of soaking.

Since the soak water was always drained off and discarded before the samples were transferred to rag dolls, amylase activity in this discarded portion was determined. The methods used indicated that beta-amylase activity was absent and that only a trace of alpha-amylase activity could be detected. The pregermination soak resulted then in an absorption of water, no significant change in beta-amylase, and some increase in alpha-amylase.

TABLE II
WEIGHT CHANGES AND AMYLASE DEVELOPMENT IN WHEAT DURING THE
PREGERMINATION SOAK

Treatment	Total green weight	Total dry weight	Beta-amylase activity		Alpha-amylase activity	
			"Free"	Total	"Free"	Total
	g	g	units	units	units	units
Untreated	30.0	26.7	10.1	28.4	0.027	0.043
12-hour soak	39.4	27.4	9.7	28.9	0.035	0.070
24-hour soak	45.8	26.9	10.6	28.8	0.029	0.056
12-hour germination	47.2	27.7	10.7	29.3	0.097	0.122

Saccharogenic activity: Curves indicating the development of saccharogenic activity during germination at the four temperatures are shown in Figure 1. It may be noted from Table I that in the instances of germination at 20°, 15°, and 10°C the total saccharogenic activity showed an increase during the initial period, followed in turn by a slight decrease and then a steady increase. The significance of these minor variations is questionable and to prevent confusion in the graphing such initial deviations are not shown by the curves of Figure 1. Likewise the same behavior may be noted in connection with the free extracts of those samples germinated at 5°C. Here, too, the variation is of questionable significance and was ignored in drawing the curve.

The data, as recorded in Figure 1, clearly illustrate the manner in which saccharifying activity, both free and total, increases slowly during the initial stages and then at an accelerated rate as the seedling develops. This is true at all four germination temperatures. The amounts of free and total activity approach each other and are approximately equal at the level of about 40 units. This level was reached at about 4, 6, 10, and 24 days respectively for the four germination temperatures of 20°, 15°, 10°, and 5°C.

The data of Figure 1 are in agreement with the findings of Weichherz and Asmus (1931) and Myrbäck and Myrbäck (1933) that the free and total saccharogenic activities of germinating barley increased steadily and became approximately equal in the later stages of germination. They do not agree with the findings of Effront (1905), Wald-

schmidt-Leitz and Purr (1931), and Lüers and Rummeler (1933) for barley, and Naylor and Dawson (1936) for oats, that saccharifying power decreases in the later stages of seedling development. The enzymatic changes responsible for the development of saccharogenic activity are best illustrated by a consideration of the individual amylase components.

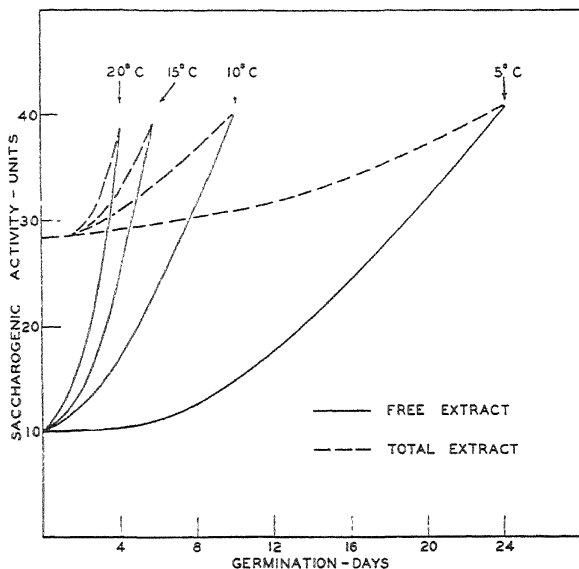


Fig. 1. Saccharogenic activities in the "free" and total extracts of wheat germinated at temperatures of 20°, 15°, 10°, and 5°C.

Beta-amylase activity: The data relative to the development of beta-amylase at the four germination temperatures is shown graphically in Figure 2. The amount of papain-extractable beta-amylase increased slightly in the initial growth stage. This increase was followed by a decline resulting in the activities of the final samples being in all cases below that of the original wheat sample. More striking was the progressive increase in the amounts of free beta-amylase. At the end of the germination periods the free beta-amylase was equal to the total; *i.e.*, there was about three times as much free beta-amylase as originally present. The results of germination at the four temperatures were very similar, about the only difference being that beta-amylase development took place at a decelerated rate as the temperature of germination was lowered.

The interpretation of the small change in activity shown by the total beta-amylase and the rapid increase in "free" beta-amylase to eventually coincide with the total is obvious. As germination progresses the "bound" beta-amylase (that fraction not readily extractable by water) is liberated by the action of the proteolytic enzymes simultaneously formed (Myrbäck and Myrbäck, 1933; Hills and Bailey, 1938a) until a point is reached at which none remains in the bound state.

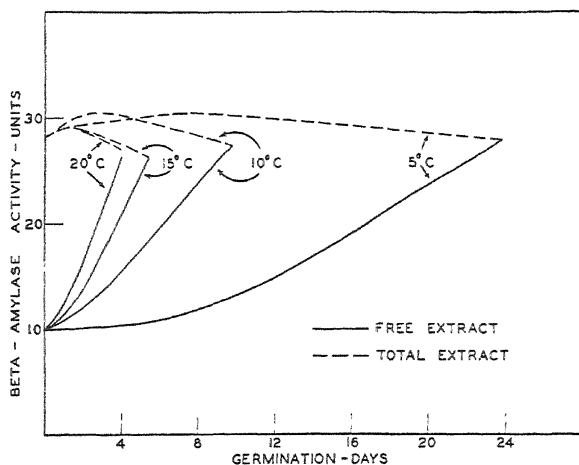


Fig. 2. Beta-amylase activities in the "free" and total extracts of wheat germinated at temperatures of 20°, 15°, 10°, and 5°C.

No other data are available which record accurate measurements of beta-amylase. However, acid inactivation of the alpha component has been used by several workers in an attempt to isolate the action due to beta-amylase. Using this technique Lüers and Rümmler (1935) and Ugrumow (1935), working respectively with barley and wheat, reported that the initial increase of "beta-amylase" was not maintained in the later stages of seedling growth. The results as recorded in Figure 2 do not indicate such a leveling off in activity for the free beta-amylase but conform more closely with the data reported by Ohlsson and co-workers (1932-1938) for germinating cereals.

Alpha-amylase activity: The development of alpha-dextrinogenic activity in germinating wheat is illustrated by the data recorded in Figure 3. Only the results found for the activities of the total extracts are shown. The data of Table I indicate that the developments of "free" and total alpha-amylase are essentially parallel phenomena.

Too, the extraction techniques used resulted in the free extracts' exhibiting nearly as much alpha-amylase activity as the total; the three-hour period used for the water extractions apparently permitted sufficient proteolytic activity to liberate most of the "bound" alpha-amylase. Shorter extractions were used later with a few of the samples and a much wider spread between water- and papain-extractable alpha-amylase was found.

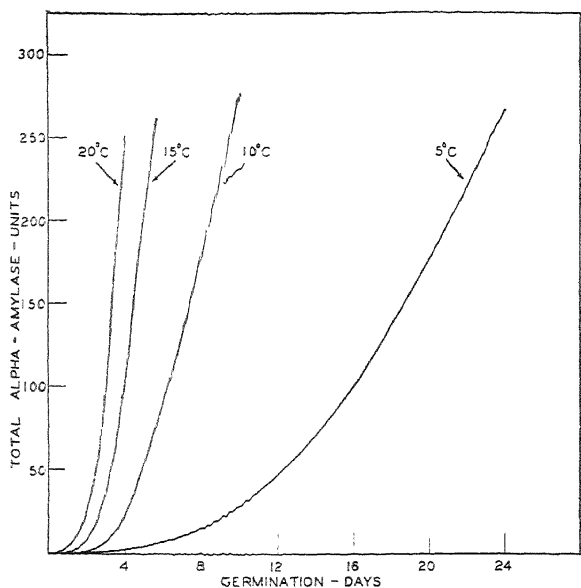


Fig. 3. Alpha-amylase activity in the total (papain) extracts of wheat germinated at temperatures of 20°, 15°, 10°, and 5°C.

The curves of Figure 3 are somewhat similar to those resulting from the free beta-amylase data recorded in Figure 2. After an initial slow increase in alpha-amylase the rate of development is greatly accelerated. Most workers have reported that alpha-amylase does not appear until after a period of germination ranging from one to several days. Application of the method sensitive to very small amounts of alpha-amylase indicates that such development progresses from the very beginning of germination (Figure 3 and Table I). Chrzaszcz and Janicki (1933) also were able to detect a steady increase in dextrinizing enzyme through the first stages of the development of barley seedlings.

So-called alpha-amylase methods used previously in studies of germination have not been specific for the alpha component (Sandstedt,

Kneen, and Blish, 1939). However, the development of "liquefying activity," shown by Kneen, Beckord, and Sandstedt (1941) to be essentially synonymous with alpha-amylase activity, has been studied by Effront (1905), Lüers and Rümmler (1933), and Chrzaszcz and Janicki (1933). Too, the development of that portion of the alpha-dextrinogenic activity remaining after heat inactivation of the beta component has been studied by Ohlsson and co-workers (1932-1938), Ugrumow (1935), and Lüers and Rümmler (1935). The progressive increase in alpha-amylase throughout seedling development shown by the data as recorded in Figure 3 is in fair agreement with the findings of these authors.

The nature of the increase of alpha-amylase activity resulting from papain extraction of barley malt has been attributed by Hills and Bailey (1938a) to an activation of the alpha component already present

TABLE III
EFFECT OF PEPTONE, BOTH DURING AND FOLLOWING EXTRACTION,
ON ALPHA-AMYLASE ACTIVITY

Extract and treatment	Alpha-amylase activity
	<i>units</i>
Papain extract	66.7
Peptone extract—"Difco"	56.1
Peptone extract—"Witte"	57.1
Water extract	50.0
Water extract + "Difco" peptone	50.0
Water extract + "Witte" peptone	50.0

in solution rather than a liberation of "bound" enzyme. This conclusion was based chiefly on the finding that peptone stimulated the dextrinizing activity of a solution of alpha-amylase. To investigate this hypothesis several different types of extraction of a malt were made as well as attempts to activate the alpha-amylase in a "free" extract. All extractions were for 30 minutes and with the same ratio of malt to extraction liquid (1 g malt plus 100 ml of a solution containing 1 mg calcium acetate per 1 ml. of water). Two commercial peptones were used, "Difco" and "Witte." In the "peptone extracts" peptone was added to the malt meal before extraction at the rate of 50 mg per gram of malt. For the "papain extract" papain was added to the dry meal at the rate of 100 mg per gram of malt. Where peptone was added to the "free" extract concentrations were adjusted to the same level as used for extraction. The results are shown in Table III.

The data of Table III indicate that the two peptones were approximately alike in their effects. Their presence during extraction resulted in an extract of somewhat greater alpha-amylase activity than the

"free" but considerably less than that of the papain extract. This lends support to the finding of Chrzaszcz and Janicki (1933) that peptone extracts had higher amylase activity than water extracts. However, the data indicate that this increase in alpha-amylase activity by peptone extraction is a liberation of alpha-amylase and not an activation of the enzyme already present in solution. Neither brand of peptone had any effect on alpha-amylase activity when added to the water extract. The present data indicate then that, as with beta-

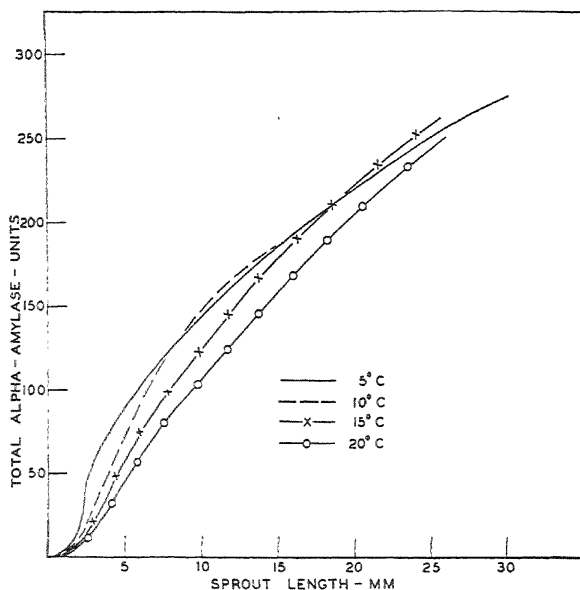


Fig. 4. Relationship between alpha-amylase activity and sprout length of wheat germinated at temperatures of 20°, 15°, 10°, and 5°C.

amylase activity, the action of papain is to liberate a fraction of alpha-amylase that is resistant to the solvent action of water.

Sprout elongation and alpha-amylase development: It may be seen from Table I that the appearance of sprouts is preceded by a considerable development of alpha-amylase. As growth progresses this enzymic development roughly parallels sprout elongation. This is shown by the data as illustrated in Figure 4. It is also apparent that there is a temperature relationship. Particularly in the early stages of seedling development, the lower the germination temperature the higher the amount of active alpha-amylase per millimeter of sprout length. For example the processes leading to the development of 5 mm of sprout

length resulted in twice as much alpha-amylase at 5°C as at 20°C. It should be remembered of course that a much greater time was required for the production of both sprouts and enzyme at the lower temperature. In the later stages of germination the temperature effect was minimized and seedlings with equal sprout lengths were approximately equal in alpha-amylase activity.

Absolute changes in enzyme content: It has been pointed out above that very little loss in total dry weight was caused by germination at either 15° or 10°C. However, the weight loss during the 4 days at 20°C was considerable. Amylase activity was calculated per gram of

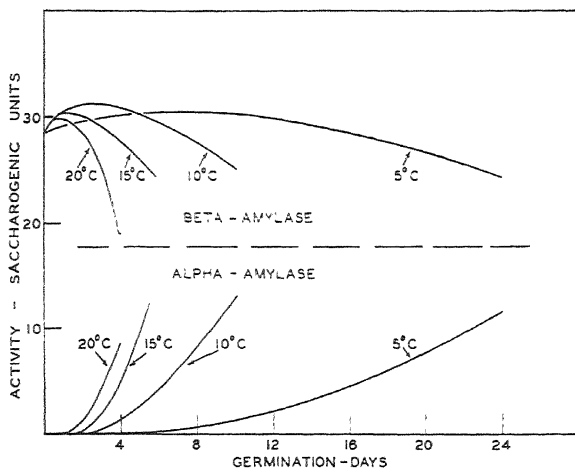


Fig. 5. Absolute changes in the contents of total beta- and alpha-amylase in wheat germinated at 20°, 15°, 10°, and 5°C. (Activities of beta components are calculated in terms of saccharifying power.)

dry germinated grain and as such is indicative of an absolute gain or loss only in cases where the total dry weight of the germinated sample was identical with that of the original ungerminated wheat sample. Accordingly the data for the total alpha- and beta-amylase in the seedlings were recalculated on the basis of the dry weight of the original grain. Such values should then be indicative of absolute changes in the amounts of these enzymes.

The data illustrating absolute changes are shown in Figure 5. Alpha-amylase activity, as well as beta-amylase activity, is expressed in terms of saccharogenic units. These "alpha-saccharogenic" units were calculated by subtracting beta-saccharogenic units from malt-saccharogenic units. By this means the activities of the two components were placed on a more comparable basis. As in previous

figures the curves representing total beta-amylase development do not follow the erratic results obtained in the early stages of germination but indicate the general trends.

As diagramed in Figure 5 the data show that there was an apparent increase in the amount of total beta-amylase during the initial stages of germination, followed by a pronounced and progressive decrease. Simultaneously with this decrease in beta-amylase, there was a progressive increase in the total amount of alpha-amylase present. Changes were similar at all four germination temperatures, differences being essentially a matter of rapidity.

It would appear that there must be some relationship between the loss of beta-amylase and the appearance of and increase in content of alpha-amylase. Certainly the relationship between these two changes is too close to permit the conclusion that two *unrelated* processes occur simultaneously: a loss, or inhibition, of beta-amylase and a synthesis, or activation, of alpha-amylase.

Discussion

The studies reported above possibly have little practical application in the production of malt destined for use in the brewing industry. The germination conditions differed greatly from those customarily used in malt production. Moreover, the amylase content of brewer's malt is only one of the many factors influencing malt "quality." However, amylase activity (Geddes, Hildebrand, and Anderson, 1941), and more specifically alpha-amylase activity (Kneen and Sandstedt, 1941b), is the major and probably the only factor of any great significance in the evaluation of a malt for flour supplementation.

In a separate communication (Kneen and Sandstedt, 1941b) it is suggested that malts for flour supplementation be purchased on the basis of their alpha-amylase activity. This necessitates production and marketing of such malts on a similar basis, *i.e.*, on the basis of alpha-amylase content. In other words problems involved in the production and use of malt supplement are essentially those pertaining to the most efficient production and use of malt alpha-amylase. Geddes, Hildebrand, and Anderson (1941) have demonstrated that modification of standard malting procedure may be desirable for the production of such malts. The present study was designed to provide additional information regarding the relationship of certain germination conditions to the development of amylases.

The data indicate that, with the conditions used, the most efficient production of alpha-amylase was at the intermediate temperatures of 15° and 10°C. At these temperatures very high levels of alpha-amylase coincided with a minimum weight loss. A higher temperature (20°C)

resulted in a very rapid production of the enzyme but involved considerable weight loss. Further studies to determine the conditions most suited to the practical production of malt alpha-amylase are essential.

Summary

Hard red winter wheat was germinated at four temperatures and examined periodically to investigate changes in green and dry weight, sprout length, and the saccharogenic, beta-amylase, and alpha-amylase activities of the "free" and total (papain) extracts. Changes taking place at the different temperatures were quite similar, differing essentially only in the rapidity of change. At the germination temperatures of 20°, 15°, 10°, and 5°C approximately 4, 6, 10, and 24 days respectively were required to produce equal levels of sprout length and amylase activity.

Green weight of the developing seedlings increased steadily throughout the germination period. On the other hand total dry weight showed some decrease, this decrease being most pronounced at the 20°C germination temperature.

Both free and total saccharogenic activity (per gram of dry sample) increased progressively throughout germination, the free extract becoming equal in activity to the total after seedling development had progressed to the point at which sprout length approximated 30 to 35 mm. Total beta-amylase activity showed a slight increase during the first stages of growth, followed by an effective decrease. Increase in free beta-amylase was progressive throughout, the free and total activities becoming essentially equal in the later stages. Alpha-amylase activity, both free and total, increased steadily with seedling growth, in this respect essentially paralleling increase in sprout length.

When calculations were based on the original dry weight of the sample it was found that there was a very considerable decrease in the amount of total beta-amylase through the later stages of growth, this decrease being coincident with a similar increase in total alpha-amylase. In addition to changes taking place in the amylolytic activities of the "free" extracts, in part attributable to the action of the proteolytic enzymes developing simultaneously, there was then an apparent loss or inhibition of beta-amylase and a synthesis or activation of alpha-amylase. The suggestion is advanced that these changes may be closely related.

The significance of the data as applied to the production of malt for flour supplementation is discussed. Since the alpha-amylase content of a malt apparently is the only factor of any appreciable significance in supplementation it would seem advisable to so regulate malting practice as to bring about the most efficient production of this enzyme.

Literature Cited

- Brown, H. T., and Morris, G. H.
1890 Researches on the germination of some of the Gramineae. *J. Chem. Soc. (London)* 57: 458-528.
- Chrzaszcz, T., and Janicki, J.
1933 Die wirkliche und scheinbare Amylasemenge in verschiedenen Getreidearten vor und während Keimung. *Biochem. Z.* 265: 260-281.
- Duddleston, B. H.
1920 The modified rag doll and germinator box. *Purdue Univ. Agr. Exp. Sta. Bul.* 236.
- Effront, Jean
1905 Sur le développement de l'amylase pendant la germination des grains. *Compt. rend.* 141: 626-628.
- Ford, J. S., and Guthrie, J. M.
1908a Contribution to the biochemistry of barley. I. The amylase of resting barley. *J. Inst. Brewing* 14: 61-87.
1908b The amylolytic and proteolytic ferments of wheaten flours, and their relation to "baking value." *J. Soc. Chem. Ind. (London)* 27: 389-393.
- Gäumann, Ernst
1932 Der Einfluss der Keimungstemperatur auf die chemische Zusammensetzung der Getreidekeimlinge. I. *Z. Botan.* 25: 385-461.
- Geddes, W. F., Hildebrand, F. C., and Anderson, J. A.
1941 The effect of wheat type, protein content, and malting conditions on the properties of malted wheat flour. *Cereal Chem.* 18: 42-60.
- Hills, C. H., and Bailey, C. H.
1938a The nature of the increase in amylase activity of germinating barley. *Cereal Chem.* 15: 273-281.
1938b The amylases of different barley varieties. *Cereal Chem.* 15: 351-362.
- Kneen, Eric, Beckord, O. C., and Sandstedt, R. M.
1941 The starch-degrading properties of barley malts. *Cereal Chem.* 18: 741-754.
— and Sandstedt, R. M.
1941a Beta-amylase activity and its determination in germinated and ungerminated cereals. *Cereal Chem.* 18: 237-252.
1941b The evaluation of malt for use as a flour supplement. *Cereal Chem.* (in press).
- Lintner, C. J.
1887 Studien über Diastase. II. *J. prakt. Chem. N.S.* 36: 481-498.
- Lüers, Heinrich
1936 Changes in enzymes during malting. *Cereal Chem.* 13: 153-171. Also *Wochschr. Brau.* 52: 249-254 (1935).
- and Lechner, R.
1933 Einwirkung von Papain auf Gerste und Malz und deren Auszüge. *Wochschr. Brau.* 50: 33-38.
- and Rümmler, W.
1933 Die Entwicklung der Amylase während der Keimung der Gerste. *Wochschr. Brau.* 50: 297-300.
1935 *Ibid.* 52: 9-12.
- Myrbäck, K., and Myrbäck, S.
1933 Über Gersten- und Malzamyase. *Biochem. Z.* 258: 158-171.
- Naylor, N. M., and Dawson, V. L.
1936 Effect of treatment of oats on the development of saccharogenic and dextrinogenic amylases during germination. *Iowa State Coll. J. Sci.* 10: 267-274.
- Nordh, G., and Ohlsson, Erik
1932 Amylasen in ruhenden und keimenden Samen. I. Gerste. *Z. physiol. Chem.* 204: 89-100.
- Ohlsson, Erik
1926 On the two components of malt diastase. *Compt. rend. trav. lab. Carlsberg* 16 (No. 7): 1-68.
1930 Über die beiden Komponenten der Malzdiastase, besonders mit Rücksicht auf die Mutarotation der bei der Hydrolyse der Stärke gebildeten Produkte. *Z. physiol. Chem.* 189: 17-63.

- and Edfeldt, Olle
1933 Amylasen in ruhenden und keimenden Samen. III. Hafer. *Z. physiol. Chem.* 221: 174-179.
- and Thorn, Nils
1938 Amylasen in ruhenden und keimenden Samen. V. Die Entwicklung der Alpha-Amylase der keimenden Gerste. *Compt. rend. trav. lab. Carlsberg. Sér. chim.* 22: 398-404.
- and Uddenberg, C. E.
1933 Amylasen in ruhenden und keimenden Samen. II. Roggen. *Z. physiol. Chem.* 221: 165-173.
- Sandstedt, R. M., and Kneen, Eric
1941 The determination of small quantities of alpha-amylase (in manuscript).
- Kneen, Eric, and Blish, M. J.
1939 A standardized Wohlgemuth procedure for alpha-amylase activity. *Cereal Chem.* 16: 712-723.
- Snider, S. R.
1941 The determination of total beta-amylase in barley and barley malt. *Cereal Chem.* 18: 186-200.
- Stenstam, T., Björling, C. O., and Ohlsson, Erik
1934 Amylasen in ruhenden und keimenden Samen. IV. Weizen. *Z. physiol. Chem.* 226: 265-271.
- Ugrumow, P. S.
1935 Alpha- und Beta-amylase in reifenden Weizensamen. *Biochem. Z.* 282: 74-78.
- Waldschmidt-Leitz, E., and Purr, A.
1931 Über Amylokinase, einen natürlichen Aktivator des Stärkeabbaus in keimender Gerste. *Z. physiol. Chem.* 203: 117-131.
- Weichherz, J., and Asmus, R.
1931 Untersuchungen über die enzymatischen Vorgänge bei der keimenden Gerste. I. Die Entwicklung der diastatischen Kraft. *Biochem. Z.* 237: 20-72.

AMYLASE AND PROTEASE SYSTEMS OF MALTED WHEAT FLOUR¹

F. C. HILDEBRAND and G. M. BURKERT

General Mills, Inc., Research Laboratories, Minneapolis, Minnesota

(Read at the Annual Meeting, May 1941)

Amylase Activity

Malt products have long been used as baking supplements because of their property of increasing gas production in fermenting doughs. This stimulation of fermentation has been ascribed to the enhanced amylase activity of such malt products. In the instance of barley malt, it was early determined that at least two amylase factors were present. These were designated originally (Kuhn, 1925) as alpha- and beta-amylase because of the property of the first enzyme to form the alpha-glucoside and of the second to produce the beta modification of maltose. Ohlsson (1926) and Nordh and Ohlsson (1932) developed a procedure for separating the two amylase components that was based on the acid-lability of alpha-amylase and the thermolability of beta-amylase.

¹ Paper No. 29, Journal Series, General Mills, Inc., Research Laboratories.

These components were found to differ markedly in the manner of their action on the starch substrate, which, in this case, was a gelatinized starch paste. It was found that alpha-amylase apparently ruptured the starch molecule at more or less central linkages, producing degradation products of relatively high molecular weight and bringing about concomitant rapid decrease in viscosity and loss of the characteristic blue color with iodine and the property of being precipitated by 60% aqueous alcohol. Each of the three last-mentioned characteristics has been utilized by various investigators as a means of following alpha-amylase activity.

Beta-amylase was found characteristically to act on starch so as to form hydrolysis products predominantly of low molecular weight. In the presence of beta-amylase, it has been found that there is a relatively rapid increase in reducing power, and the blue coloration with iodine characteristic of undegraded starch persists for a much longer period than in the instance of the action of alpha-amylase. An excellent review of these and other considerations involving the amylase systems of cereals has been given by Hanes (1937).

Applying Ohlsson's technique for amylase separation, Andrews and Bailey (1934) showed that in flours milled from normal wheat, maltose production is due primarily to the action of beta-amylase. These authors further demonstrated that during germination, alpha-amylase is produced or activated, and malted wheat flours are accordingly a good source of this factor.

More recent studies on the amylase systems of malted wheat flour have been carried out largely by Blish, Sandstedt, and their co-workers. In 1937, Blish, Sandstedt, and Mecham confirmed the findings of Andrews and Bailey and advanced evidence indicating the presence of a third factor, predominantly occurring in malted wheat, which had the property of attacking raw starch. Fractionation studies with ammonium sulfate, using both boiled and raw starches as substrates, showed that this "raw starch factor" and alpha-amylase were not identical although the two were found to be very similar in their properties.

It is now believed that the efficacy of malted wheat flour in increasing gas production in fermenting doughs is due to hydrolysis of high-molecular-weight starch particles by alpha-amylase or the raw-starch factor with the formation of dextrans of relatively low molecular weight. These dextrans are in turn hydrolyzed by beta-amylase, of which there is apparently an excess in all flours, thus providing an adequate supply of maltose for yeast fermentation. The work of Blish, Sandstedt, and Mecham has also suggested the presence in malted wheat flour of a nonenzymic inhibitor and a nonenzymic "activator."

Protease Activity

An exhaustive study of the proteolytic activity of malted wheat flours has been made by Mounfield (1936a, 1936b, 1938). In these studies Mounfield, using edestin as a substrate and following the extent of proteolysis by determination of the increase in free amino or carboxyl groups by the Sørensen formaldehyde titration, found that sprouted wheat contained an enzyme capable of hydrolyzing the whole protein at an optimum pH of 4.1 at 40°. He also demonstrated the presence of a dipeptidase capable of hydrolyzing leucylglycine and glycylglycine at an optimum pH of 7.5. Both enzymes were found to be spon-

TABLE I
PROTEASE ACTIVITY OF MALTED AND UNMALTED WHEAT AND WHEAT FLOUR

Material	Proteolytic activity	
	Unactivated	Activated
DATA OF BALLS AND HALE (1936)		
Whole wheat flour (a)	1.95	2.40 ¹
Whole wheat flour (b)	0.70	1.15 ²
Wheat malt	3.00	3.15 ²
White flour	0.45	0.75 ²
DATA OF HILDEBRAND (1939)		
Patent flour, A mix	0.3	
1st clear, A mix	0.9	
2nd clear, A mix	3.6	
Patent flour, B mix	0.6	
1st clear, B mix	1.2	
2nd clear, B mix	4.8	
Malt flour (a)	2.0	
Malt flour (b)	6.2	
Ground wheat malt	3.9	

¹ Glutathione activation.

² Cysteine activation

taneously inactivated on standing in aqueous solution at room temperature.

It was found that during the process of germination the proteinase activity of wheat increased approximately six times in four days and ten times in seven days, that the spontaneous inactivation found during storage in aqueous suspension could be materially reduced by adding glycerol to a 45% concentration, and that both enzymes were activated by cyanide. In the last respect these findings are in agreement with the work of Balls and Hale (1936), who have shown that the proteases of wheat are activated by reducing substances.

Mounfield (1938) demonstrated that results obtained with edestin as the substrate could not be quantitatively applied where either gluten or a mixture of glutenin and gliadin were used. In sharp contrast to

the effect previously noted by the same author and to the studies of Balls and Hale, no activating effect of cyanide could be found when gluten was used as substrate.

Quantitative data on the proteolytic activity of malted wheat flours are relatively meager. In Table I are shown typical results taken from the studies of Balls and Hale (1936) and Hildebrand (1939). Since the data were obtained by two different methods of determination, they may not be inter-compared, but they will serve to show the relative activity of malted wheat and malt flours compared to that of unmalted wheat and flour. Balls and Hale, measuring the increase in amino groups by alcoholic titration, found that wheat malt had approximately seven times the protease activity of patent flour and from two to three times that of whole-wheat flour. The values given in the lower section of Table I, obtained by the rate-of-gelation procedure

TABLE II
PROTEINASE ACTIVITY OF MALTED AND UNMALTED WHEAT FLOURS

Sample	Proteinase activity (as mg nonprotein N/100 g)		
	No yeast	3% yeast	3% yeast + octyl alcohol
Patent flour A	117	94	(98)
Patent flour B	48	70	(28)
Patent flour C	31	50	(26)
Malt flour A	257	310	348
Malt flour B	233	276	301
Malt flour C	317	336	378

of Landis and Frey (1938), indicate that malt flour is from three to twenty times as active as patent flour but may be lower in activity than a second clear flour produced from unmalted wheat.

Similar comparisons may be made from the data in Table II, first column. These values, obtained by the Ayre and Anderson (1939) trichloroacetic acid precipitation method, indicate that malt flours are from 2.5 to 5 times as active as patent flours. The data shown are typical of a large number of determinations carried out on flours from both unmalted and malted wheats.

In Table II is also shown the effect of the presence of yeast on proteolytic activity. When 3% of yeast based on the weight of flour was included in the autolytic digests, there was a fairly regular increase in production of nonprotein nitrogen, as shown by the data in the second column. These values, however, are undoubtedly too low because of the utilization of some nonprotein nitrogen by the respiring yeast. Freilich and Frey (1942) suggested the use of octyl alcohol to

inhibit yeast respiration. The data in the third column of Table II show the results obtained in the presence of both yeast and octyl alcohol. In the instance of malted wheat flours, it is evident that there is an apparent further increase in the production of nonprotein nitrogen, and it is believed that these last figures are a true measure of proteolytic activity of such material in fermenting doughs. The data obtained with patent flour under these conditions, however, are open to question. It was exceedingly difficult to obtain reproducible results under these conditions, and there seems to be no adequate explanation for the apparent large decrease in nonprotein nitrogen here noted.

Effect of Malted Wheat Flour on Dough Softening

It has been shown repeatedly that the addition of excessive amounts of malted wheat flour to doughs causes increased mobility and, with extended fermentation periods, may give rise to excessive stickiness. Early workers were of the belief that these effects were due entirely to protein hydrolysis, but in 1933 Kosmin advanced the theory that the production of sticky doughs and moist crumb was due to liquefaction and dextrinization of starch. Brief experiments led to her belief that the hydrolytic changes in gluten structure were not of sufficient magnitude to account for the effects noted. This theory has received increasing support from other investigators in succeeding years. Thus Read and Haas found that malted wheat flours had a relatively low proteolytic activity compared to that of other proteases and therefore concluded that the sticky quality of doughs containing high dosages of malted wheat flour must be attributed to alpha-amylase. In 1936, the same authors treated aqueous extracts of various proteolytic agents with safranin according to the technique proposed by Marston (1923) in order to remove proteases. They found that malt preparations thus treated and presumably, therefore, free from proteases were still able to cause stickiness in doughs.

Bohn and Bailey (1937) in studying changes in physical properties of doughs found that the addition of malted wheat flour appreciably lowered stress readings. Although doughs prepared with 1% and 2% malted wheat flour were found to be more sticky and mellow than fermented doughs with 0.5% malt, differences in physical behavior were not detectable by either stress or five-minute farinograph measurements. These authors state: "It has not been proven that the softening effect is entirely due to the papainase enzyme present in flour. It is possible that other enzymes, proteolytic, diastatic, or otherwise, may also be partly responsible for the mellowing effect. It is difficult to obtain pure proteolytic enzymes free from amylases and *vice versa*, and thus the problem as to what enzyme or enzymes

cause the softening effect upon the gluten or dough system remains unsolved."

Munz and Bailey (1937) obtained alpha- and beta-amylase preparations from malted wheat flours by the Ohlsson technique. The protease content of these preparations was reduced by precipitation with safranine. Using the farinograph to follow changes in dough consistency, it was found that increase in mobility was obtained only in preparations containing alpha-amylase. When this enzyme was inactivated by acid treatment, extracts exerted no significant effect on dough properties.

Sandstedt, Jolitz, and Blish (1939) concluded that stickiness in doughs is associated with the presence of amyloextrins produced by the action of alpha-amylase, lending further support to the belief that alpha-amylase or some factor associated with it in malted wheat flour is responsible for stickiness.

Geddes, Hildebrand, and Anderson (1941), in a study of the effect of malting conditions on the properties of malted wheat flour, utilized a series of experimentally produced malted wheat flours having a fairly wide range of proteolytic activity. However, when such flours were added to a common untreated base flour in amounts sufficient to give constant and adequate gas production, the amount of proteinase activity contributed by the malted wheat flours was a very small fraction of the total activity in the blends. In no case could as much as 5% of the total activity be ascribed to the malted wheat flour. However, in the experiments described above, the experimental malting conditions were not varied as widely as possible, and, at the time the proteolytic activity determinations were carried out, the malted wheats had been in storage for an extended period. While it was believed that such storage would not affect relative results, it does cast some doubt on the reliability of the absolute values found.

In connection with another problem, the authors have had available a series of 48 experimentally produced malted wheat flours exhibiting a wide range in proteinase activity. In order to check the results reported by Geddes, Hildebrand, and Anderson, the amount of each of these flours required to give a constant and adequate gas production was determined and a series of blends comprising the required amount of each malted wheat flour, with a common untreated base flour, was prepared. Gas production determinations were made by the method described by Sherwood, Hildebrand, and McClellan (1940), and it was found that the blends gave a mean gas production of 147 ml of CO₂ from the second to the fifth hour of fermentation, with a standard deviation of ± 2.0 ml. Calculation of the proteinase activity contributed by the malted wheat flours showed that this amount ranged

from 0.08 to 4.0 mg of nonprotein nitrogen per 100 g, whereas the base flour employed (patent flour *A* in Table II) contributed 117 mg per 100 g. It is obvious, therefore, that even under the most unfavorable circumstances the amount of proteinase activity that can be ascribed to added malted wheat flour is an extremely low percentage of the total.

In spite of the apparent low proteolytic activity due to malted wheat flour in these blends, it was deemed desirable to ascertain whether or not the variation in such proteolytic activity would exert an appreciable effect on dough-handling properties and/or baking characteristics. To this end, the 48 blends described above were baked in duplicate in random order by the basic A.A.C.C. procedure with the exceptions that a four-hour fermentation period was employed in order to accentuate any differences that might be found, and 5% sugar was included in the formula to insure adequate gas production under this extended fermentation period. No evidence whatever could be found of significant variations in loaf volume, crumb, or crust characteristics or dough-handling properties among the doughs thus baked. Accordingly, it must be concluded that, while the proteolytic activity contributed by the malted wheat flours under these conditions varied from approximately 0.1 to 4 mg of nonprotein nitrogen, such variation was totally without effect on baking properties.

The experiments described above have been supplemented with other studies designed to ascertain the factor responsible for dough softening in the presence of excessive treatments of malted wheat flour. Malted wheat flour of relatively high amylase activity and low proteinase activity was extracted and the extract treated with safranine according to the technique described by Marston (1923). The latter author states that separation of proteinase is complete only in the absence of protein degradation products. The extract was accordingly tested for amylase and proteinase activity, both before and after treatment with safranine. It was found that the extract had the same capacity for stimulating gas production as did an equivalent weight of the original malted wheat flour, and accordingly it was assumed that quantitative extraction of alpha-amylase and/or the raw starch factor described by Blish, Sandstedt, and Mecham had been attained.

In order to determine the effect of safranine treatment on the proteolytic activity of this extract, equal portions of the extract before and after safranine treatment were allowed to act on casein for one- and three-hour periods. The extent of nonprotein nitrogen production was determined by the usual technique, and it was found that treatment with safranine had reduced proteolytic activity by approximately one-third. On this basis, the proteolytic activity of this extract when

acting on flour protein was also assumed to have been reduced to this extent.

To 300-g portions of an untreated base flour were added increasing amounts of this extract, 3% yeast, and sufficient water to give the proper mobility. The doughs were mixed in the farinograph, removed, and allowed to ferment for four hours at 30°. At the end of this period, 450-g aliquots of each dough were scaled and remixed. It was noted that with increasing increments of malted wheat flour, there was a progressive increase in stickiness of the doughs and a progressively greater increase in mobility between the original doughs and those remixed after four hours of fermentation. The results obtained are given in Table III. The addition of safranine precipitate equivalent to 5% of malted wheat flour did not give a significant alteration in dough consistency or stickiness.

TABLE III
EFFECT OF SAFRANINE-TREATED EXTRACT OF MALTED WHEAT FLOUR ON
DOUGH MOBILITY

Extract added, as malt flour equivalent	Proteinase activity due to		Fraction of total PA due to extract	Increase in mobility in 4 hrs at 30°
	Extract	Base flour		
%	mg nonprotein N		%	Brabender units
0	0	360	—	175
1	7.4	360	2.0	210
2	14.8	360	3.9	200
5	37.0	360	9.3	235
10	74.0	360	17.1	300
Proteinase ppt. equiv. to 5% malted wheat flour	55.2	360	13.3	185

The results described above tend to substantiate the belief previously advanced that the increase in stickiness and in dough mobility noted with excessive treatment of malted wheat flour cannot be ascribed to proteolytic activity of such flours but are due rather to excessive alpha-amylase activity.

Summary

Brief reviews of the present state of our knowledge of the amylase and protease systems of malted wheat flours have been given.

Evidence is advanced to support the belief that increase in dough mobility and stickiness noted with the addition of excessive dosages of malted wheat flour is to be ascribed to alpha-amylase activity contributed by such flours rather than to proteinase activity.

Acknowledgment

The authors wish to thank P. M. Sautier, D. K. Mecham, and B. A. McClellan for their assistance in these studies.

Literature Cited

- Andrews, J. S., and Bailey, C. H.
1934 The amylases of normal and germinated wheat flours. *Cereal Chem.* 11: 551-556.
- Ayre, C. A., and Anderson, J. A.
1939 Varietal differences in barleys and malts. VI. Autolytic proteolytic activity of malt and its correlations with wort nitrogen and barley nitrogen fractions. *Can. J. Research* 17C: 239-246.
- Balls, A. K., and Hale, W. S.
1936 Proteolytic enzymes of flour. *Cereal Chem.* 13: 54-60.
- Blish, M. J., Sandstedt, R. M., and Mecham, D. K.
1937 Action of wheat amylases on raw wheat starch. *Cereal Chem.* 14: 605-628.
- Bohn, L. J., and Bailey, C. H.
1937 Effect of fermentation, certain dough ingredients, and proteases upon the physical properties of flour doughs. *Cereal Chem.* 14: 335-348.
- Freilich, J., and Frey, C. N.
1942 Dough oxidation and mixing studies. V. Correlations between protease activity, reducing matter, and oxidizing effects in dough. *Cereal Chem.* (in press).
- Geddes, W. F., Hildebrand, F. C., and Anderson, J. A.
1941 The effect of wheat type, protein content, and malting conditions on the properties of malted wheat flour. *Cereal Chem.* 18: 42-60.
- Hanes, C. S.
1937 The action of amylases in relation to the structure of starch and its metabolism in the plant. *New Phytologist* 36: 101-141, 189-239.
- Hildebrand, F. C.
1939 A comparison of methods for the determination of proteolytic activity. *Cereal Chem.* 16: 792-798.
- Kozmin, Natalie
1933 Biochemical characteristics of dough and bread from sprouted grain. *Cereal Chem.* 10: 420-436.
- Kuhn, Richard
1925 Der Wirkungsmechanismus der Amylasen; ein Beitrag zum Konfigurationsproblem der Stärke. *Ann.* 443: 1-71.
- Landis, Q., and Frey, C. N.
1938 A sensitive method for the determination of proteolytic activity. *Cereal Chem.* 15: 91-101.
- Marston, H. R.
1923 The azine and azonium compounds of the proteolytic enzymes. *Biochem. J.* 17: 351-359.
- Mounfield, J. D.
1936a The proteolytic enzymes of sprouted wheat. *Biochem. J.* 30: 549-557.
1936b The proteolytic enzymes of sprouted wheat. II. *Biochem. J.* 30: 1778-1786.
1938 The proteolytic enzymes of sprouted wheat. III. *Biochem. J.* 32: 1675-1684.
- Munz, E., and Bailey, C. H.
1937 Effect of the enzymes of malted wheat flour upon certain properties of flour and dough. *Cereal Chem.* 14: 445-457.
- Nordh, G., and Ohlsson, E.
1932 Amylasen in ruhenden und keimenden Samen. I. Gerste. *Z. physiol. Chem.* 204: 89-100.
- Ohlsson, Erik
1926 On the two components of malt diastase. *Compt. rend. trav. lab. Carlsberg* 16: 1-68.
- Read, J. W., and Haas, L. W.
1936 Further studies on the baking quality of flour as affected by certain enzyme actions. Relative starch liquefying power of the diastatic agents investigated. *Cereal Chem.* 13: 14-37.
- Sandstedt, R. M., Jolitz, C. E., and Blish, M. J.
1939 Starch in relation to some baking properties of flour. *Cereal Chem.* 16: 780-792.
- Sherwood, R. C., Hildebrand, F. C., and McClellan, B. A.
1940 Modification of the Bailey-Johnson method for measurement of gas production in fermenting dough. *Cereal Chem.* 17: 621-626.

NUTRITIVE VALUE OF THE PROTEIN AND MINERAL CONSTITUENTS OF RICE VARIETIES

A. SREENIVASAN

Department of Biochemistry, Indian Institute of Science, Bangalore

(Received for publication February 18, 1941)

Extensive feeding experiments with pigeons, on the antineuritic values of several samples of Indian rice, both raw and parboiled, have been carried out by McCarrison and Norris (1924). Spruyt (1930) has reported that his colorimetric method of estimating vitamin B₁ in rice samples gives results in complete accord with those obtained by feeding the samples of rice to rice birds (*Munia* birds). Aykroyd (1932), using the method of biological assay of Chick and Roscoe (1929), has investigated the comparative vitamin B₁ contents of raw and parboiled rices, polished to different degrees. The biological value of the proteins of rice has been determined by a few workers (McCollum and Simmonds, 1917; Osborne and Mendell, 1918; Mitchell and Villegas, 1923; Mitchell and Carman, 1924, 1926; Morgan, 1931; Swaminathan, 1937; Basu and Basak, 1937; and others) and it has been recorded that rice, in spite of its somewhat low protein content, has a biological value higher than that of most other cereals, including wheat.

Apart from these studies, very little attention seems to have been directed towards following comparative rates of growth, in young rats or pigeons, when fed different varieties of rice as the sole source of proteins and minerals in an otherwise adequate diet. It was therefore decided to investigate the possibility of such varietal differences.

Experimental

Series I: Six varieties of rice, Adt 3, Co 9, Co 10, Adt 7, GEB 24, and Adt 11, were chosen for this experiment. These showed somewhat similar tendencies in regard to protein and mineral variations. The powdered samples of the (unpolished) rices were steamed in the dry state at 120°C. in an autoclave for 2½ hours on two successive days. This period was sufficient to inactivate the antineuritic vitamin present in the samples.

Composition of rice varieties: Table I gives the protein and mineral contents of the varieties of rice (shelled, unpolished) used in this and following experiments.¹ The methods of analysis were those of the A.O.A.C. (1935).

¹ These samples were obtained through the kind courtesy of the Government Paddy Specialist, Coimbatore, Madras.

Technique of feeding: It was found that cooking the rice grains before feeding did not supply a good food, as the material tended to set into a hard horny mass on partial drying, thus making it very difficult to be used by the rats. Besides, a good portion of the feed was scattered by the animals when fed in this form. Hence, in these experiments, the rice was powdered and with the other supplements, made into a sort of cake. This was done by making a fairly thick paste of the flour with water and pouring it on a hot plate well smeared with arachis oil. Sufficient quantity of food in the form of cake was given to the different rats.

To the rice samples, autoclaved in the dry powdered condition at 120°C., vitamin B₁ was supplied from an external source.

TABLE I
COMPOSITION OF RICE SAMPLES (OVEN-DRY BASIS)

Variety	Protein	Total mineral	P ₂ O ₅	CaO
Adt 6	8.67	1.85	1.28	0.10
Adt 3	10.47	1.62	0.82	0.12
Co 9	9.59	1.76	0.73	0.10
Co 10	7.61	1.65	0.79	0.06
Adt 7	7.26	1.70	0.56	0.13
GEB 24	6.97	1.66	0.54	0.07
Adt 11	6.59	1.46	0.70	0.09

Groups of four to six young rats,² about 30 days old, were given sufficient quantities of the rice powders in the form of cakes, together with the same supplements of cod-liver oil (0.05 g. daily) and marmite extract (2 cc. of a 5% extract, daily). The latter were uniformly smeared on the cakes so as to keep their proportions in the different diets more or less constant.

Growth charts were maintained for the different rats in each of the six groups for a period of seven weeks. At about this period the rate of growth began to decline in all the groups; in a few cases, the animals did not appear quite healthy and their fur dropped gradually. Since the diets consisted mainly of rice, the protein level of the food was only between 6.6% and 10.5%; obviously, this was not optimal for growth over prolonged periods. Hence, a small supplement of casein (3% by weight of the diet) was now given and the experiments continued for another seven weeks. It was not assumed that a supplement of this order would introduce additional variables in the experiment. The average growth

² Sometimes unequal numbers of animals had to be used for the reason that sufficient young rats were not always available, especially when 20 or more rats were required at one time. In other experiments where the same number of rats were used for each group to start with, there were occasional deaths in the middle of the experiment. It has however been recorded in the text of the paper wherever such deaths occurred.

curves for the different groups are given in Figure 1, while the growth rates are presented in Table II.

The results have been examined statistically by Fisher's analysis of variance (1935). The weekly average increase in weight for the different groups ranged approximately between 4 and 7 g. with the varieties. The average rate for the period before the casein supplement was given was invariably more than for the entire 14 weeks. This may be due to the fact that the rate of growth in young rats, even with inadequate diets, is greater during the first few weeks and falls down with

TABLE II
EFFECTS OF VARIETAL FACTORS ON AVERAGE WEEKLY GROWTH RATE (SERIES I)

Variety of rice	Number of rats used	Average weekly increase in weight in g. during		Remarks			
		7 weeks	14 weeks				
Adt 3	5	7.49	6.00	One rat died on 69th day.			
Co 9	4	6.96	5.67	One rat died on 74th day.			
Co 10	5	5.97	5.00	One rat died on 61st day.			
Adt 7	4	4.75	4.29	One weighed maximum 86 g. (stunted) and died on 88th day.			
GEB 24	5	4.88	4.36	—			
Adt 11	6	4.10	3.79	One rat died on 58th day and another on 79th day.			
Standard error per sample: 0.27 0.26							
Significance:							
Without casein		<u>Adt 3</u>	<u>Co 9</u>	<u>Co 10</u>	<u>Adt 7</u>	<u>GEB 24</u>	<u>Adt 11</u>
With casein		<u>Adt 3</u>	<u>Co 9</u>	<u>Co 10</u>	<u>Adt 7</u>	<u>GEB 24</u>	<u>Adt 11</u>

age. It may be noted however that, between the varieties, the order in which growth rates decreased significantly was more or less the same with and without the casein supplement.

In another report (Sreenivasan and Sadasivan, 1941), the comparative nutritive values of the protein and mineral constituents of three varieties of rice grown under (a) dry, (b) swamp, broadcast, and (c) transplanted, swamp conditions were studied by the same technique as above. The effect on growth of the protein and mineral contents of these samples and the six varieties above (Table II) were examined together and it was found that the average growth rates due to the different rices depended upon their contents of proteins (x_1), phosphorus compounds (x_2), and minerals excluding phosphate (x_3). Each of these was correlated with the growth rate (y) during the precasein period of seven weeks by means of a regression equation:

$$y = 0.42x_1 + 7.34x_2 + 3.43x_3.$$

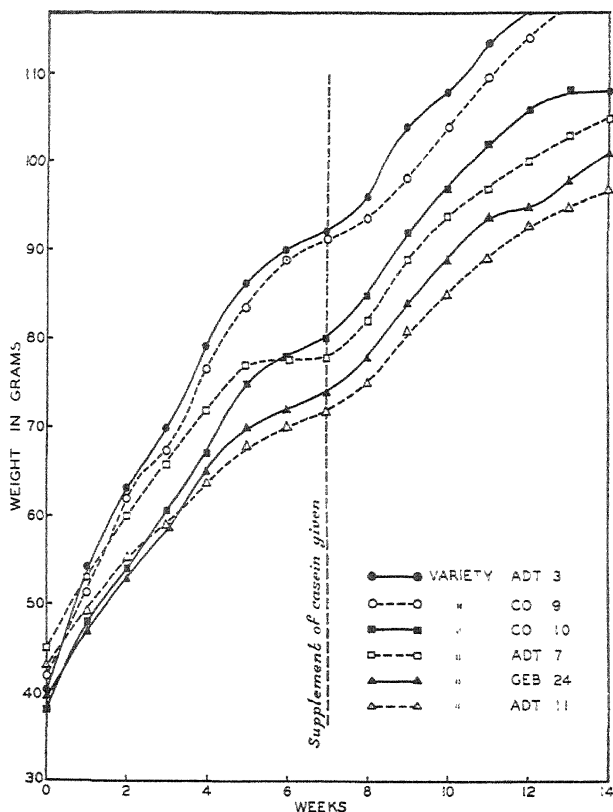


Fig. 1. Average growth rate, Series I.

The values of partial regression coefficients were:

	<i>S.E.</i>	<i>t</i>	<i>n</i>	<i>P</i>
$b_1 = 0.42 \pm 0.05$		8.4	61	0.01
$b_2 = 7.34 \pm 1.28$		5.7	61	0.01
$b_3 = 3.43 \pm 0.57$		6.0	61	0.01

The value of the multiple correlation coefficient R was $+ .9232$ and was significant on the 1% level (Table III).

Series II: The protein levels of the diets used in the above series were somewhat low as compared to the normal requirements of rats. Besides, the animals did not receive any supplements of minerals other than what was contained in the rice varieties themselves. Since it was observed that growth was better correlated with phosphorus than with

TABLE III
TEST OF SIGNIFICANCE OF MULTIPLE CORRELATION COEFFICIENT

Variance due to	Degrees of freedom	Sums of squares	Mean square	Ratio of variances	Remarks
Regression	3	80.5800	26.8600	117.1902 ¹	Significant
Deviation	61	13.9809	0.2292	—	—
Total	64	94.5609	—	—	—

¹ Significant on 1% level.

the total minerals, it was considered desirable to study the effect of supplementing the rice diets with phosphate-free salt mixture on growth. Accordingly, in the following series, the nutritive values of two rice samples (GEB 24 and Co 9) were compared exactly as above, using groups of five animals for each rice. But the animals received in addition a daily supplement of 5% of the rice as a phosphate-free salt mixture composed of calcium lactate (150 parts), magnesium sulfate (30 parts), ferric citrate (12 parts), sodium chloride (10 parts), and potassium chloride (10 parts).

TABLE IV
EFFECTS OF MINERAL SUPPLEMENTS ON AVERAGE WEEKLY GROWTH RATE
(SERIES II)

Variety of rice	Average weekly increase in weight in grams during	
	7 weeks	14 weeks
Co 9	8.43	6.52
GEB 24	7.14	5.60
Standard error per sample	0.30	0.21

Periodic observations on growth rates were made as before and, when the growth rate began to decline at the end of 6 to 8 weeks, casein supplement (3%) was given to the diets and the experiments continued for a further equal period (Table IV and Fig. 2).

The differences in growth rates between the varieties were significant and confirmed the earlier observations in regard to the relationship between growth and nitrogen and phosphorus contents. It may be noted that addition of the salts has increased the growth rate in this series as compared to series I.

*Series III:*³ The foregoing experiments have shown that, given adequate supply of the antineuritic and other vitamins, the growth-promoting qualities of different varieties of rice are essentially related

³ A note embodying the results of this series has since been published by Sreenivasan (1939).

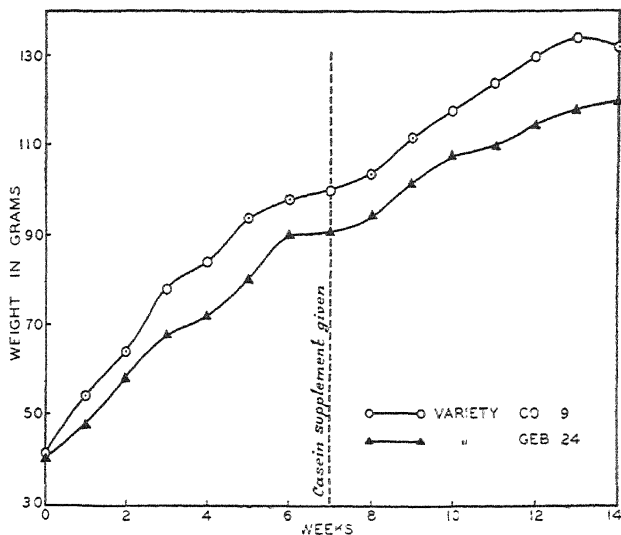


FIG. 2. Average growth rate Series II.

to their nitrogen and mineral (particularly phosphorus) contents. In the present series, an attempt has been made to compare the growth-promoting qualities of the phosphorus compounds of two of the varieties, Adt 6 and Adt 11. The powdered, autoclaved rices were used for feeding, but in one case (Adt 11) the rice flour was intimately mixed with 2% of a preparation of rice protein (purity 94%) so that the initial protein contents of the two feeds were nearly the same. The diets were then mixed with 5% of phosphate-free salt mixture as in Series II. Groups of four young rats were fed on the two samples thus prepared, together with the same supplements of cod-liver oil and marmite extract. The experiments were continued for a period of six weeks. The weekly growth increases are given in Table V.

TABLE V
AVERAGE WEEKLY GROWTH INCREASES WITH EQUAL PROTEIN LEVELS
(SERIES III)

Rice diet	Weekly increase in weight in grams					
	1st week	2nd week	3rd week	4th week	5th week	6th week
Adt 6	13.2	12.6	10.8	8.4	7.0	6.2
Adt 11 + 2% rice protein	9.6	10.4	8.4	6.8	5.4	5.0

Standard error per sample: 0.26

TABLE VI
AVERAGE GROWTH RATES OF PIGEONS WITH DIFFERENT RICE DIETS

Group	Treatments in diet	Pro- tein	P ₂ O ₅	Initial weight	Final weight	Weekly increase in wt.	Remarks
1	Unpolished (Co 9), autoclaved + polishings from Co 9	10.10	0.80	219	255	7.2	—
2	Unpolished (Adt 11), autoclaved + polishings from Co 9	7.82	0.78	222	249	5.4	—
3	Polished (Co 9), autoclaved + polishings from Co 9	8.36	0.64	218	248	6.0	One bird died on 16th day.
4	Polished (Adt 11), autoclaved + polishings from Co 9	7.36	0.52	225	246	4.2	One bird died on 18th day and another on 30th day.
5	Unpolished (Co 9), autoclaved + polishings from Adt 11	8.55	0.89	220	251	6.2	—
6	Unpolished (Adt 11), autoclaved + polishings from Adt 11	6.27	0.87	226	251	5.0	—
7	Polished (Co 9), autoclaved + polishings from Adt 11	6.81	0.73	220	243	4.6	One bird died on 22nd day.
8	Polished (Adt 11), autoclaved + polishings from Adt 11	5.77	0.61	215	233	3.6	—
9	Unpolished (Co 9), control	8.42	0.64	218	250	6.4	—
10	Unpolished (Adt 11), control	5.77	0.61	219	238	3.6	One bird died on 22nd day.
Standard error per sample: 0.22							
Significance groups: <u>1</u> <u>9</u> <u>5</u> <u>3</u> <u>2</u> <u>6</u> <u>7</u> <u>4</u> <u>8</u> <u>10</u>							

The differences between the varieties were highly significant. Thus, in spite of the protein contents having been adjusted to the same level in both the diets, there was a significant difference in growth rates due to the two varieties of rice. This is evidently traceable to the differences in the mineral contents of the grains. Since the diets contained enough supplements of all the minerals except phosphorus, it is reasonable to assume that the better growth induced by Adt 6 was due to its higher phosphorus content which was nearly 1.8 times that of Adt 11.

Experiments with pigeons: Ten groups of young pigeons, four each, were fed for a period of 35 days on two varieties (Co 9 and Adt 11) of rices, unpolished as well as polished, and subjected to different treatments (Table VI). One of the varieties (Co 9) was a coarse-grained red rice, while the other was a medium-grained white rice. Polishing

with each variety was done until the bran was just completely removed from the grain. Where polishings were added to the food, this was done in the proportions in which they existed in the original unpolished rices.

The diets were designed to throw light on the comparative nutritive qualities of the varieties in relation to their mineral, protein, and vitamin B₁ contents. For feeding the birds, the rice grains were cooked in an autoclave with the necessary amounts of water and then mixed with the requisite quantities of bran from red or white rice, together with small doses of cod-liver oil (0.05 g. per bird) and autoclaved marmite extract (2 cc. of 5% extract per bird). Feeding was done *ad libitum*. Growth records were maintained for all the pigeons throughout the period of experiment. Table VI gives the different treatments, the protein and phosphate contents of the diets and the weekly average growth rates of the pigeons in the different groups.

The experiments could not be continued over a longer period as, in most cases, the pigeons began to go down in weight and show signs of ill health. Besides, they showed an aversion to the food. It was therefore concluded that the diets were not palatable and perhaps also did not supply optimal quantities of nutritional ingredients.

The results show that growth, in general, is related to the protein and phosphorus contents; grain colour as such had probably no effect. It appeared that the amounts of vitamin B₁ supplied by the rice polishings, in quantities used in these experiments, were inadequate for the birds since head retraction appeared in a few cases after about 30 days. It must be noted however that, since the feeds were predominantly starchy in character, they would, in consequence, require larger amounts of vitamin B₁ for proper metabolism.

Discussion

Young rats fed on a diet in which rice was the only source of protein grew slowly, especially after the first few weeks and showed signs of fur dropping. Growth was stimulated by supplementing the diet with casein. Suzuki, Matsuyama, and Hashimoto (1925) have demonstrated that rats fed on a diet containing rice protein never weighed more than 130 g.; standard growth was obtained only by employing 15% or more of rice protein in the diet. Similar results on the inadequacy of rice as the sole source of protein in diets given to rats have been reported by Hermano (1933), who found that, under such conditions, the animals were stunted and did not weigh more than 104 g. In other experiments carried out by the present author (unpublished data) it has been observed that with diets containing over 90% of rice young rats require

larger feed per unit increase in weight. With pigeons, low-protein diets, although taken in normal quantities during the first few weeks, were not well liked by the birds. They took less food on continued feeding with such diets and showed definite indications of digestive disorders.

Though a rice diet does not continue to maintain growth over prolonged periods, it must yet be borne in mind that, in ordinary practice, rice does not serve as a large proportion of the diet and is generally a part of a good mixed diet, which includes pulses and vegetables. There is no doubt that, under such conditions, rice will have a high nutritive value. Recent work at Bangalore (Ranganathan and Rau, 1938) has shown that the biological value of the proteins of rice in a mixed diet was greatly increased by the addition of calcium. Supplements of skimmed milk or calcium lactate have also been shown to enhance greatly the nutritive value of a rice diet (Wilson, Ahmad, and Mullick, 1936; Aykroyd and Krishnan, 1937). Further work on the effect of other supplements to make rice a complete and well-balanced food needs to be carried out.

The differences in growth rate due to the varieties of rice examined are due to differences in protein and mineral contents. There is a general and widespread belief that early-maturing varieties of rice are inferior to late-maturing ones (Chambliss and Adams, 1915; Wells, Agcaoilli, and Feliciano, 1922; Acton and Chopra, 1925; Basu and Sarkar, 1935; and others). While it is true that long-season crops invariably yield better, there are yet many short-season varieties of rice which contain greater percentages of albuminoid and mineral matter (Sadasi- van and Sreenivasan, 1938).

Since the nutritive values of rice varieties are mainly determined by their composition, it would follow that there is great need for improving quality in rice along the lines of enriching its composition by selection of suitable varieties and by proper manuring. Cultural methods and irrigation are important factors in improving not only yield but also the quality of the proteins and minerals as well (Kelley and Thompson, 1910; Wells *et al.*, 1922; Sadasivan and Sreenivasan, 1938).

The increased growth rates observed with addition of phosphate-free salt mixture (Series II) would show that proper supplements of minerals will enhance the nutritive value of rice. Rice contains between 1% and 2% of mineral matter with phosphorus as a major component. The phosphorus occurs mostly as phytin in organic combination (Rather, 1918; McCance and Widdowson, 1935) and, in this form, is not considered as readily available to the animal organism (McCance and Widdowson, 1935; Lowe and Steenbock, 1936). The results of the present experiments (Series III) would show however that with the same level

of protein supply, the variety of rice which is high in phosphorus induces better growth in rats. It cannot therefore be said that the phosphorus in rice is entirely unavailable. It is likely that the culinary conditions or the possible presence of an intestinal phytase in the rat (Patwardhan, 1937) renders at least part of the phosphorus compounds in rice assimilable. The results indicate the need for further work on the ingestion of phosphorus in rice under different conditions, and on the effect of calcium and of the calcium : phosphorus ratio on the availability of the phytin in rice.

Summary

Groups of young albino rats fed different varieties of rice after autoclaving, and with supplements of cod-liver oil and marmite extracts only showed variations in growth rates that were essentially related to protein and mineral contents of the rices. The rates of growth decreased after about two months when the animals attained a weight of 90 to 100 g. Supplements of casein given at this stage resulted in increased growth which, in the different groups, was again proportional to the nitrogen and mineral contents of the rices.

The correlation between rate of growth during seven weeks and the proteins, phosphorus compounds, and minerals other than phosphates has been shown by means of a regression equation.

The weekly average growth rate of young rats fed on rice diets increased when a supplement of phosphate-free salt mixture was given. The differences due to varieties were still of the same order.

In experiments with rice diets containing the same level of protein but with different amounts of phosphorus, it was observed that the growth rate was greater with the variety of rice containing the greater percentage of phosphorus.

Similar results on the relationship between growth and composition of the feeds were obtained in experiments with groups of pigeons fed on two varieties of rice, unpolished as well as polished and with and without the addition of rice polishings.

The significance of these observations in relation to the nutritive values of the protein and mineral constituents of rice varieties and the availability of phytin in rice is discussed.

Acknowledgments

The author desires to express his grateful thanks to Mr. N. C. Datta, M.Sc., for much valuable help, to Professor V. Subrahmanyam for his kind encouragement, and to Rao Bahadur M. Vaidyanathan, M.A., L.T., F.S.S., for suggestions in the statistical presentation of the data. Thanks are also due to the Imperial Council of Agricultural Research for a research grant during the tenure of which this work was carried out.

Literature Cited

- Acton, H. W., and Chopra, R. N.
 1925 Epidemic dropsy and beriberi. *Indian Med. Gaz.* 60: 1-18.
- Aykroyd, W. R.
 1932 The effects of parboiling and milling on the antineuritic vitamin (B_1) and phosphate content of rice, *J. Hyg.* 22: 184-192.
- and Krishnan, B. G.
 1937 The deficiencies of the South Indian diet. *Indian J. Med. Research* 24: 1093-1115; 25: 367-372.
- Association of Official Agricultural Chemists
 1935 Methods of analysis.
- Basu, K. P., and Basak, M. N.
 1937 Biological value of the proteins of Aman and Austices and of the polishes. *Indian J. Med. Res.* 24: 1043-1066.
- Basu, K. P., and Sarkar, S. N.
 1935 The chemical composition of various rice samples. *Indian J. Med Research* 22: 745-758.
- Chambliss, C. E., and Adams, E. L.
 1915 The culture of rice in California. U. S. Dept Agr. Farmers' Bul. 688
- Chick, H., and Roscoe, M. H.
 1929 A method for the assay of the antineuritic vitamin B_1 . *Biochem. J.* 23: 498-503.
- Fisher, R. A.
 1935 Statistical methods for research workers.
- Hermano, A. J.
 1933 The nutritive protein value of five varieties of rice. *Philippine J. Sci* 51: 567-572.
- Kelley, W. P., and Thompson, A. R.
 1910 Hawaii Agr. Exp. Sta. Bul. 21
- Lowe, J. T., and Steenbock, H.
 1936 Cereals and rickets. VII. The role of inorganic phosphorus in calcification on cereal diets. *Biochem. J.* 30: 1126-1134
- McCance, R. A., and Widdowson, E. M.
 1935 Phytin in human nutrition. *Biochem. J.* 29: 2094-2099.
- McCarrison, R., and Norris, R. V.
 1924 The relationship of rice beriberi in India. *Indian Med Research Memoirs*, No. 2
- McCollum, E. V., and Simmonds, N.
 1917 A biological analysis of pellagra-producing diets. III. The value of some seed proteins for maintenance. *J. Biol. Chem.* 32: 347-368.
- Mitchell, H. H., and Carman, G. G.
 1924 The biological value for maintenance and growth of the proteins of whole wheat, eggs, and pork. *J. Biol. Chem.* 60: 613-620.
 1926 The biological value of the nitrogen of mixtures of patent white flour and animal foods. *J. Biol. Chem.* 68: 183-215.
- and Villegas, V.
 1923 The nutritive value of the proteins of coconut meal, soy beans, rice bran and corn. *J. Dairy Sci.* 6: 222-242.
- Morgan, A. F.
 1931 The effect of heat upon the biological value of cereal proteins and casein. *J. Biol. Chem.* 90: 771-792.
- Osborne, T. B., and Mendel, L. B.
 1918 Nutritive factors in plant tissues. I. The protein factor in the seeds of cereals. *J. Biol. Chem.* 34: 521-532.
- Patwardan, V. N.
 1937 The occurrence of a phytin-splitting enzyme in the intestines of albino rats. *Biochem. J.* 31: 560-564.
- Ranganathan, V., and Rau, Y. V. S.
 1938 Effect of addition of calcium on the biological value of the proteins of Indian diets. *Nature* 142: 165.

- Rather, J. B.
1918 The nature of the inosite phosphoric acids of some important feeding materials. *J. Am. Chem. Soc.* **40**: 523-536.
- Sadasivan V., and Sreenivasan, A.
1938 Studies on quality in rice. II. Chemical composition of rice varieties. *Indian J. Agr. Sci.* **8**: 807-818.
- Spruyt, J. P.
1930 Investigation on a colorimetric method for estimating the silverskin (antiberiberi vitamin) percentage of rice. *Chem. Weekblad* **27**: 298-304.
- Sreenivasan, A.
1939 Availability of phosphorus in rice. *Nature* **143**: 244-245.
- and Sadasivan, V.
1942 Nutritive value of the protein and mineral constituents of dry and wet cultivated rices. *Cereal Chem.* **19**: (following paper, this issue).
- Suzuki, U., Matsuyama, Y., and Hashimoto, N.
1925 Relative nutritive value of various proteins contained in Japanese food articles. *Inst. Phys. Chem. Research* **4**: 1-47.
- Swaminathan, M.
1937 The relative value of the proteins of certain foodstuffs in nutrition. *Indian J. Med. Research* **24**: 767-786; **25**: 57-79.
- Wells, A. H., Agcaouli, F., and Feliciano, R. T.
1922 Philippine rice. *Philippine J. Sci.* **20**: 353.
- Wilson, H. E. C., Ahmad, B., and Mullick, D. N.
1936 *Indian J. Med. Research* **24**: 161.

NUTRITIVE VALUE OF THE PROTEIN AND MINERAL CONSTITUENTS OF DRY AND WET CULTIVATED RICES

A. SREENIVASAN and V. SADASIVAN

Department of Biochemistry, Indian Institute of Science, Bangalore

(Received for publication February 15, 1941)

Perhaps the most important among the requirements of the rice plant is an abundant supply of fresh water, either rain or irrigation, during the major part of the growing season. The crop stands partly submerged in the field for over two-thirds of the growing period. Although the rice plant flourishes best under swampy conditions, there are still certain dry cultivated rices which are grown in certain parts of India and elsewhere. These do not require puddling and prolonged swamping in the same way as the commoner varieties do. Irrigated rice is generally more productive than dry cultivated rice. Even those varieties of rice that are cultivated under dry-land conditions yield more if irrigated.

Dry-land cultivated rices are however popularly believed to be more nutritious than wet-land cultivated rices. According to McCarrison (1928), the conditions of cultivation of rice, especially in regard to water supply during the growth of the crop, are factors of importance in determining the endemicity of beriberi. He has adduced experimental evi-

dence to show that the lower nutritive value of rice grown under wet-land conditions is due in part at least to its lower content of vitamins of the water-soluble B class. If this is generally the case, it would be desirable to extend the method of dry cultivation and, if possible, improve on it so as to combine superior quality with high yield. The problem therefore appeared to merit further study and accordingly the present inquiry was undertaken.

Experimental

Three different varieties of rice, Dirty modan, Black modan, and Katta modan, were grown during April to September, 1936, at the Agricultural Research Station, Pattambi, West Coast, with identical manurial treatments (10 cartloads of cattle manure *plus* 2,000 pounds of wood ash per acre) but under different conditions of cultivation as follows: (1) irrigated, transplanted, (2) irrigated, broadcast, and (3) dry, broadcast. In (1) and (2) the fields were maintained in the puddled condition in

TABLE I
EFFECTS OF DIFFERENT ENVIRONMENTS AND CULTURAL METHODS ON
YIELD OF PADDY IN POUNDS PER ACRE

	Dirty modan			Black modan .			Katta modan		
	Dry broadcast	Wet broadcast	Trans-planted	Dry broadcast	Wet broadcast	Trans-planted	Dry broadcast	Wet broadcast	Trans-planted
Sowing date	23-4	16-5	16-5	23-4	16-5	16-5	15-5	16-5	16-5
Transplanting date	—	—	10-6	—	—	10-6	—	—	10-6
Flowering date	10-7	28-7	2-8	10-7	28-7	2-8	5-8	5-8	5-8
Harvesting date	4-8	20-8	4-9	4-8	26-8	4-9	4-9	4-9	4-9
Duration, days	103	96	111	103	102	111	110	111	111
Rainfall from sowing to harvest, in.	69.6	76.8	79.8	69.6	76.8	79.8	80.9	79.8	79.8
Yield, lbs. per acre	824	1,133	1,400	783	716	1,434	740	1,267	1,734

the usual way, but in (1) the seedlings were raised in separate seed beds and transplanted at the end of 26 days, while in (2) the seeds were broadcast in the fields. In (3), the seeds were also broadcast on receipt of pre-monsoon showers but the soil was maintained in the dry condition, the crop being thus only rain-fed. While the type of land used for the two wet trials was the same, the land used for the dry trial was comparatively poor in fertility, being situated on unbunded hill slopes. This could not be avoided.

Table I gives the yields of paddy at harvest for the different samples, together with other details of the experiment.

It may be observed that, with the exception of Black modan the yield was lower under conditions of dry cultivation than with the puddled soil

(cf. Sreenivasan, 1936). In the latter case, transplanted rice invariably gave a higher yield than the same variety broadcast (cf. Howard, 1924). For strict comparison, it would have been better if the sowings had all been done on the same date. As it is, it is difficult to explain the low yield of Black modan from the wet broadcast crop as compared to the dry crop. It must be noted that the yields are only from single plots rather than from replicated experiments.

Chemical composition: The chemical compositions of the shelled, unpolished rices from the different samples were determined according to methods outlined in an earlier publication (Sadasivan and Sreenivasan, 1938). Table II gives the results.

TABLE II
COMPOSITION OF DRY AND WET CULTIVATED RICES
Percentages on fresh basis.

Mode of cultivation	Moisture	Proteins (crude)	Total minerals	P ₂ O ₅	Ether extractives
	%	%	%	%	%
DIRTY MODAN					
Transplanted, swamp	12.0	10.77	1.470	0.713	2.33
Broadcast, swamp	12.5	9.45	1.412	0.640	2.41
Broadcast, dry	11.3	8.17	1.236	0.642	2.75
BLACK MODAN					
Transplanted, swamp	11.8	10.26	1.460	0.730	2.23
Broadcast, swamp	11.8	9.61	1.434	0.700	2.35
Broadcast, dry	11.3	8.35	1.266	0.639	2.60
KATTA MODAN					
Transplanted, swamp	12.4	10.00	1.474	0.695	2.23
Broadcast, swamp	11.4	9.06	1.314	0.690	2.32
Broadcast, dry	11.4	7.87	1.334	0.624	2.66

The results show that, in every case, irrigated transplanted rice was the richest in proteins and minerals, while dry rice generally contained the lowest percentages of these constituents, broadcast irrigated rice being intermediate in composition between the two. A reverse relationship appears to hold in regard to the ether extractives of the different specimens.

Growth experiments: Feeding experiments with groups of young rats, four to five each, were carried out with the above rice samples with a view to finding out the rates of growth induced by them. The

technique of feeding was the same as that described in the previous publication (Sreenivasan, 1942). The animals, 30 to 35 days old, were given sufficient quantities of the test samples of rice (autoclaved) in the form of pan cakes, together with small uniform doses of cod-liver oil (0.05 g. daily) and marmite (2 cc. of 5% extract daily). Periodic growth charts were maintained for each animal in the different groups.

TABLE III
AVERAGE WEEKLY GROWTH RATES

Group	Mode of cultivation	Number of rats used	Average weekly increase in wt. in g. during		Remarks
			7 weeks	14 weeks	
			g.	g.	
DIRTY MODAN					
1	Transplanted, swamp	4	8.14	6.50	—
2	Broadcast, swamp	5	6.86	5.43	—
3	Broadcast, dry	5	5.86	4.64	One rat died on 62nd day.
BLACK MODAN					
4	Transplanted, swamp	4	7.43	6.07	One rat died on 18th day.
5	Broadcast, swamp	4	6.71	5.36	—
6	Broadcast, dry	4	5.43	4.36	—
KATTA MODAN					
7	Transplanted, swamp	3	7.00	5.64	—
8	Broadcast, swamp	4	6.28	5.00	—
9	Broadcast, dry	4	4.71	3.79	One rat died on 76th day and another on 92nd day.
Standard error per sample:			0.30	0.20	
Significance groups:					
Without casein			<u>1</u>	<u>4</u>	<u>7</u> <u>2</u> <u>5</u> <u>8</u> <u>3</u> <u>6</u> <u>9</u>
With casein			<u>1</u>	<u>4</u>	<u>7</u> <u>2</u> <u>5</u> <u>8</u> <u>3</u> <u>6</u> <u>9</u>

After a period of seven weeks, when the rates of growth began to decline, the protein level of the food was slightly raised by adding 3% by weight of the diets as light white casein and the experiments continued for another seven weeks. It was assumed that such a supplement will not materially affect the differences in growth rates due to the rice samples. The average weekly growth rates are given in Table III and the growth charts in Figures 1 to 3.

The number of test animals used could not be increased, owing to practical difficulties. In a few cases, some of the animals had died during the experiment. Their weights were excluded from the calculations, as deaths were accidental. The results have been examined by Fisher's analysis of variance and the differences were found to be highly significant.

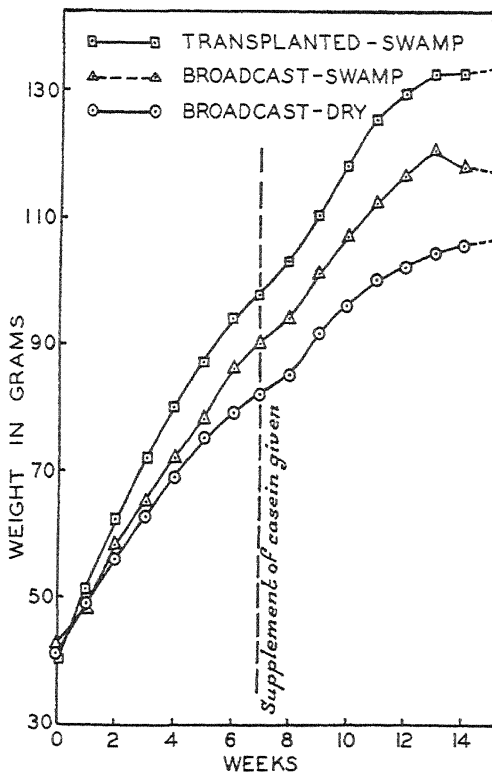


Fig. 1 Average growth rate. Dirty median variety.

It may be seen that, for any particular variety, there is a significant difference in the growth-promoting qualities of the swamp and dry cultivated rices. The transplanted swamp rice had a definitely higher nutritive value than the transplanted broadcast rice, while the dry-grown rice had the lowest nutritive value. The differences were similar both before and after the casein supplements were given. These results are in accord with the chemical composition (Table II) and support the earlier

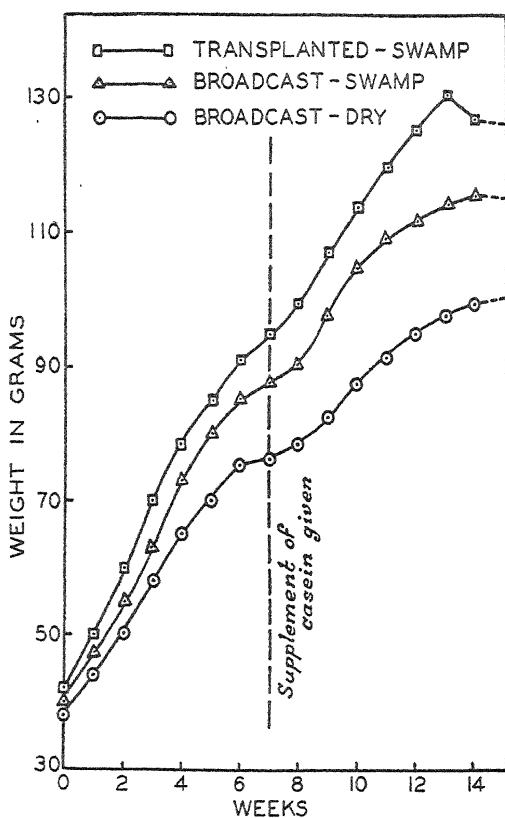


Fig. 2. Average growth rate, Black modan variety.

observation that growth is essentially related to the chemical composition, particularly the protein and mineral contents (Sreenivasan, 1942).

Discussion

Independent histological examination of the above samples have been carried out by the Paddy Specialist at Coimbatore, Madras, and it has been observed that there is a significant increase in the aleurone layer in favour of the swampy sample in all the cases (Ramiah and Mudaliar, 1939). In other work carried out by the senior author of this paper, it has been shown that when the rice plant is grown in swamp and in dry soil, the grain absorbs greater quantities of mineral nutrients in the

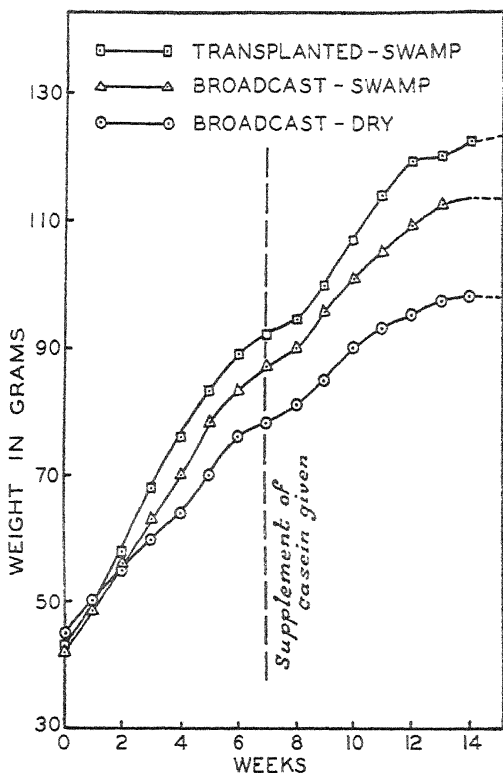


Fig 3. Average growth rate, Katta modan variety.

former case than in the latter (Sreenivasan, 1936). Not only is the total intake of nutrients more in the swamp-grown rice but also the actual percentage of the mineral constituents in the grain.

Together with the fact that the yield of paddy is also increased nearly two to three times under the conditions of the swamp soil as compared to dry cultivation, these observations would mean that wet cultivation will not only yield better but also richer grains.

These results do not seem to support the findings of McCarrison (1928). It is possible that this technique of feeding was not such as to bring out the real differences between the nutritive values of the different rice samples. Thus, in his experiments, the test samples of rice formed only 12% of a basal diet which, according to the author, "provided a sufficiency of suitable protein, fats, inorganic elements and

water" and was besides "not devoid either of vitamin A or of vitamin B." In the present investigation, however, the diets, which were fed *ad libitum*, consisted essentially of the test samples of rice and yielded the pronounced differences observed between the different groups of animals. The results do not throw any light on the relative vitamin B₁ contents of dry and wet cultivated rices, as this factor was supplied from an external source.

The results of the wet transplanted and wet broadcast crops are perhaps the only ones that can be strictly compared because of differences in sowing dates and soil conditions with the dry crop. It is clear however that irrespective of the mode of cultivation, growth is mainly determined by the composition of the rice samples.

The practical application of the present inquiry is perhaps limited because dry cultivation of rice is more a matter of necessity than of choice and the farmer will gladly forsake it—if only for greater yield—as soon as he can get a supply of water necessary for wet cultivation.

Summary

The chemical composition and nutritive value of the protein and mineral constituents of three different rice varieties grown under (a) irrigated, transplanted, (b) irrigated, broadcast, and (c) dry, broadcast conditions have been studied.

Irrigated rice, irrespective of variety, was found to be richer in protein and mineral contents than dry cultivated rice; in the former case, transplanted rice contained higher percentages of these constituents than broadcast rice. A reverse relationship holds in regard to the fat contents of the different specimens.

In experiments on the growth of young albino rats fed with the different rice samples as the sole source of proteins and minerals, it was found that dry cultivated rice was least nutritious compared to wet cultivated, transplanted rice. Irrigated broadcast rice was intermediate in nutritive value between dry and irrigated, transplanted rices.

Acknowledgments

The authors are deeply indebted to Professor V. Subrahmanyan for his keen interest and kind encouragement; to Mr. K. Ramiah, formerly Paddy Specialist, Government of Madras, for suggesting this line of work; and to Mr. C. R. Srinivasa Iyengar, formerly Superintendent, Agricultural Research Station, Pattambi, Madras, for having kindly grown the different paddy samples at his station and supplied them. The senior author is also thankful to the Imperial Council of Agricultural Research, India, for a personal grant.

Literature Cited

- Howard, A.
1924 Crop production in India. Oxford University Press.
- McCarrison, R.
1928 The influence of irrigation on the nutritive value of rice. *Indian J. Med. Research* 15: 915-920.
- Ramiah, K., and Mudaliar, C. R.
1939 The proteins in the rice grain and the development of the aleurone layer. *Indian J. Agr. Sci.* 9: 39-53.
- Sadasivan, V., and Sreenivasan, A.
1938 Studies on quality in rices. II. Chemical composition of rice varieties. *Indian J. Agr. Sci.* 8: 807-818.
- Sreenivasan, A.
1936 Effect of manuring on the growth and intake of silicon by dry and wet cultivated rice. *Proc. Indian Acad. Sci.* 3B: 258-277.
- 1942 Nutritive value of the protein and mineral constituents of rice varieties. *Cereal Chem.* 19: (previous paper, this issue).

THE RIBOFLAVIN CONTENT OF CEREAL GRAINS AND BREAD AND ITS DISTRIBUTION IN PRODUCTS OF WHEAT MILLING¹

JOHN S. ANDREWS, HAROLD M. BOYD, and DAVID E. TERRY

General Mills, Inc., Research Laboratories, Minneapolis, Minnesota

(Read at the Annual Meeting, May 1941)²

Vitamin B₂ or riboflavin is one of the important members of the B-complex required for adequate human nutrition. Although cereal grains are not abundantly endowed with this food accessory, the consumption of liberal amounts can make an appreciable contribution to the dietary requirements. The magnitude of this contribution will depend to a large extent on the form in which the cereal is consumed. It is the purpose of this paper to present some of the data which have been obtained from the authors' analyses of a variety of cereals and cereal products.

In contemplating the vitamin B₂ analyses of such materials one is confronted with the problem of selecting an adequate method. Several procedures are available, including those which depend on the growth-promoting properties of this vitamin and those which measure its color or its fluorescence in ultraviolet light. Growth promotion can be measured by the use of rats as in the method of Bourquin and Sherman (1931) or one of its modifications, by the chick method of Norris *et al.* (1936), or by the microbiological method of Snell and Strong (1939). Since growth depends on a wide variety of factors,

¹ Paper No. 33, Journal Series, General Mills, Inc., Research Laboratories.

² Subsequently to the presentation of this paper at the Omaha meeting the microbiological method was modified to eliminate certain factors which adversely affect the assay results. The data presented herein were obtained by this modified procedure.

none of these methods is entirely specific and their successful operation requires careful adjustment of other growth-affecting substances which may be present in the samples being assayed.

Colorimetric and fluorometric methods offer certain advantages over growth methods in that they are somewhat more specific. This is especially true of fluorometric procedures such as that devised by Hodson and Norris (1939). An added advantage is the rapidity with which analyses can be made. For cereals, however, these advantages are in many instances lost due to the presence of interfering materials which prevent accurate measurement of the color or fluorescence of riboflavin. Conner and Straub (1941) have recently reported on a modification of Ferrebee's method (1940), which separates the interfering substances by adsorption of the flavin on "supersorb."

The Committee on Assay of Foods of the American Public Health Association (1941) has recently reported on the status of vitamin methods. They point out the absence of any officially recognized method for riboflavin and refer to the efforts of the Association of Official Agricultural Chemists to find satisfactory procedures. Kemmerer's (1940) report on this subject describes the results of preliminary collaborative studies.

Discussion of Microbiological Method

In the present study the authors have employed the microbiological method proposed by Snell and Strong (1939). Aqueous extracts of the cereals were added to the appropriate medium and inoculated with a culture of *Lactobacillus casei*. The amount of lactic acid produced after 48 hours was taken as the measure of the riboflavin content.

The practice of using extracts of cereals for the assay is in accordance with the originally proposed method. In view, however, of the possibility that extraction may fail to remove the riboflavin completely, a preliminary study was made in which the finely divided sample was added directly to the culture medium. Under these conditions appreciably higher values were obtained suggesting that the extraction was incomplete and that assays of extracts were too low. This seemed to be confirmed by the observation that the residues remaining after extraction exhibited a small but measurable riboflavin content.

In an attempt to make the extraction more quantitative, dilute hydrochloric acid was employed and preparations of diastase were added to break down the starch components. In most cases this technique failed to give higher values than those obtained by aqueous extraction. In fact, lower values were normally obtained.

This failure to increase the riboflavin content of extracts under more drastic conditions indicates that extraction may not be a major problem in the assay method. If this is true it then becomes desirable to find another explanation for the high results obtained by direct assay of the solid material.

In carrying out the microbiological method varying amounts of the cereal or extract are added to different tubes of the culture medium. These amounts are adjusted to give growth responses which fall within the range of linearity shown by varying quantities of pure riboflavin. The flavin content is then determined by reference to the quantity of pure riboflavin required to produce the same amount of lactic acid. Values obtained for the different levels should agree within 10%.

When the direct assay is applied to cereals a linear response to different quantities sometimes fails to be observed even though the growth range falls within that exhibited by the standard flavin solutions. We have referred to this as a "falling off" phenomenon since with increasing quantities the assay values become correspondingly lower. The magnitude of the difference between the highest and lowest levels is often greater than the desired 10%.

If it is concluded that the lower results are due to the presence of substances which inhibit the growth of the organisms the higher values would then appear to be more nearly correct. Such an assumption may, however, seriously affect the results obtained with cereal grains and leads to improbably high values in the case of products such as mixed feeds. This introduces a serious question as to the validity of assuming that the higher value is the correct one.

Recovery experiments with added riboflavin do not appear to provide the answer to this problem. While frequently more than 100% recovery is obtained, this does not necessarily indicate that growth-stimulating substances are present. When flavin is added to the sample a smaller quantity must be taken for the assay in order to keep the total flavin content within the linear range of the flavin standards. Where the "falling off" phenomenon is considerable, the smaller sample will give a higher value and account for the high recovery observed. It seems possible that some cereal products may contain nonflavin substances, some of which stimulate and others which inhibit the growth of the organisms under the assay conditions. The relative concentrations of these stimulators and inhibitors may decide their relative influences on lactic acid production.

When aqueous extracts are used instead of the solid sample the "falling off" phenomenon, though still quite evident, is of considerably less magnitude while in the case of aqueous extracts which have been treated with diastase this phenomenon almost entirely disappears.

For this reason there is a preference for the use of extracts rather than the solid samples, despite the fact that lower values are obtained.

Recent studies by the authors (1941) have shown that starch has a marked stimulatory effect on the growth of *Lactobacillus casei* and that its presence in cereal extracts results in fictitiously high riboflavin analyses. In white and whole wheat flours this effect can be removed by treating their aqueous extracts with takadiastase. Riboflavin values thus obtained are very similar to those determined by fluorometric methods. This agreement between two entirely different analytical procedures greatly increases the probability that the values reported herein are substantially correct.

All the data presented in this paper were obtained from extracts prepared by autoclaving the samples with water and digesting the resulting suspensions with takadiastase. Under these conditions the "falling off" phenomenon noted above almost entirely disappears and duplicate analyses consistently agree within 10% over the entire range of the different levels employed.

Riboflavin Content of Cereal Grains

During the course of the investigations a large number of samples of whole grains was examined. One series in particular, shown in Table I, was of considerable interest since it represented six different varieties of wheat, each of which was grown at four different locations.

TABLE I
RIBOFLAVIN CONTENT OF SPRING WHEATS

Variety	Location grown				Average
	St. Paul	Morris	Waseca	Crookston	
	<i>μg per gram</i>				<i>μg per gram</i>
Renown	1.15	1.25	1.15	1.15	1.17
Rival	1.05	1.05	1.10	1.05	1.06
Pilot	1.30	1.25	1.25	1.15	1.24
Thatcher	1.25	1.20	1.20	1.20	1.21
Ceres	1.20	1.20	1.25	1.15	1.20
Marquis	1.30	1.40	1.30	1.30	1.32
Average	1.21	1.23	1.21	1.17	1.20

While the number of samples involved is too small to justify definite conclusions it is believed that certain trends are indicated. It will be observed that the averages for the different locations are practically identical, suggesting that any environmental factors at each of the stations are sufficiently constant to be without effect.

On the other hand differences occur between the averages of some of the varieties and these differences are quite similar at each station. Thus Rival contains the lowest amount of riboflavin and Marquis the highest.

These observations are in marked contrast to those reported for thiamin on the same samples (Nordgren and Andrews, 1941). The thiamin content was found to be greatly influenced by environment, while differences between the varieties were not significant.

Table II gives the results obtained on other samples of wheats.

TABLE II
RIBOFLAVIN CONTENT OF WHEATS

Description	Riboflavin	
	Average	Range
	<i>μg per gram</i>	
Soft wheats:		
15 misc. varieties	1.17	1.05-1.30
Kawvale 3 samples	1.17	1.00-1.30
Hard winter wheats:		
Turkey 7 samples	1.22	1.15-1.30
Tenmarq 7 samples	1.21	1.15-1.30
Chiefkan 6 samples	1.09	1.00-1.20
Blackhull 7 samples	1.17	1.10-1.25

The values obtained for these soft and hard winter wheats are very similar to those found in the hard spring wheats and suggest a greater constancy in riboflavin content than was found in the instance of thiamin. No effect of environment was observed and varietal differences are not particularly significant. Of the four hard winter varieties there is a trend toward lower values in Chiefkan but the magnitude of these differences is only about 10%.

A comparison of wheat with other grains can be obtained from the data given in Table III.

Barley, oats, corn, and rye are all quite similar to wheat in riboflavin content. The practically identical values obtained for the

TABLE III
RIBOFLAVIN CONTENT OF CEREAL GRAINS

Grain	Riboflavin	
	Average	Range
	<i>μg per gram</i>	
Yellow corn (13 varieties)	1.40	1.30-1.50
White corn (5 varieties)	1.38	1.30-1.50
Barley (6 varieties)	1.21	1.05-1.50
Oats (5 varieties)	1.30	1.10-1.45
Rye (6 varieties)	1.43	1.30-1.65

white and yellow corn are interesting since it is frequently assumed that the yellow varieties contain appreciably more vitamin B₂.

Riboflavin Content of Products of Wheat Milling

The products obtained from the milling of grains differ widely in their content of riboflavin, thus demonstrating the nonuniform distribution of this food accessory in the whole kernel. Table IV presents the values obtained from the analyses of the products separated in the milling of wheat.

TABLE IV
DISTRIBUTION OF RIBOFLAVIN IN PRODUCTS OF WHEAT MILLING

Mill product	Percent of wheat	Ribo- flavin	Percent of total riboflavin in wheat
	<i>%</i>	<i>μg per gram</i>	<i>%</i>
Patent flour	65.0	0.34	20.5
1st clear	5.5	0.62	3.2
2nd clear	4.5	1.85	7.7
Red dog	4.0	3.80	14.1
Shorts	12.5	2.80	32.5
Bran	8.5	2.80	22.0
Wheat	100.0	1.00	100.0

About two-thirds of the total riboflavin is contained in the feeds—red dog, bran, and shorts. The patent flour contains about one-third as much as the wheat from which it was milled. In many respects these relationships are very similar to those of vitamin B₁ except that patent flours contain a larger percentage of the total riboflavin in the wheat kernel.

No value is being reported for wheat germ since microbiological assays of this product are not yet regarded as satisfactory. The use of takadiastase in the preparation of extracts does not remove the "falling off" tendency and for this reason the assay values are probably in error.

The distribution of riboflavin in the various parts of the wheat berry is shown graphically in Figure 1. The riboflavin is represented by the solid line and for purposes of comparison the thiamin, shown by the dotted line, is also given. The similarity of the two curves suggests that the same mechanism is responsible for the way in which they are distributed throughout the wheat kernel.

Riboflavin Content of Bread

Because a large fraction of the flour milled from wheat is consumed in the form of bread it is important to examine this product, especially since some of the ingredients other than flour are important sources of

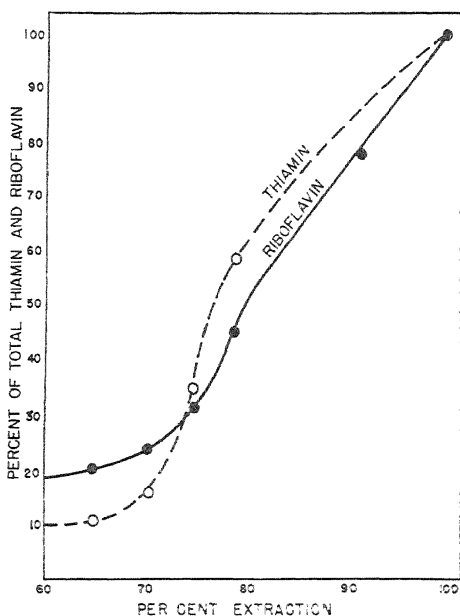


Fig. 1. Distribution of thiamin and riboflavin content in wheat.

riboflavin. Three series of breads were prepared using 2% of yeast and, in addition, salt, sugar, shortening, and yeast food. The first series was made from patent flour, the second from the same flour containing 2 μ g per gram of added riboflavin, and the third from whole-wheat flour. In each series increments of nonfat milk solids ranging from 0% to 9% were added. Table V gives the results of riboflavin assays of these products.

In many instances the agreement between the calculated and found values is extraordinarily good. Such agreement must be regarded as entirely fortuitous, since the method is not so accurate as these values might indicate.

It is apparent that there is no measurable loss of riboflavin in the production of bread. This is to be expected since flavin is quite stable to heat. On the other hand, serious losses will occur if the bread is unduly exposed to light. Early in the investigations it was found that samples of sliced bread which were allowed to dry on benches in the laboratory contained much less than the expected amount of riboflavin. Subsequent studies showed that these losses can be as high as 50% even in the absence of direct sunlight. On the other hand,

TABLE V
THE RIBOFLAVIN CONTENT OF BREADS

Bread	Calculated	Found
<i>μg per gram fresh bread</i>		
White:		
No nonfat milk solids	0.60	0.53
3% nonfat milk solids	0.99	0.92
6% nonfat milk solids	1.37	1.31
9% nonfat milk solids	1.75	1.74
White (1.27 μg of added flavin):		
No nonfat milk solids	1.84	1.78
3% nonfat milk solids	2.22	2.08
6% nonfat milk solids	2.60	2.53
9% nonfat milk solids	2.98	2.77
Whole wheat:		
No nonfat milk solids	1.02	1.16
3% nonfat milk solids	1.40	1.41
6% nonfat milk solids	1.79	1.80
9% nonfat milk solids	2.17	2.09

losses in whole wheat bread, under the same conditions of drying, are considerably less. In one experiment where a 40% loss occurred in white bread only a 10% loss was found in the whole wheat variety. It is very important in preparing bread samples for assay to carry out the drying operation in the dark.

Table VI gives the riboflavin content of the ingredients which contributed to the total in the above breads.

TABLE VI
THE RIBOFLAVIN CONTENT OF BREAD INGREDIENTS

Ingredient	Riboflavin
<i>μg per gram</i>	
Patent flour	0.35
Whole wheat flour	1.0
Yeast	31.1
Nonfat milk solids	20.6

In the case of the bread made from patent flour, the flour, 2% of yeast, and 3% of dried milk contributed appreciable amounts of riboflavin. As the amount of milk was increased its contribution became correspondingly greater. In practice the contributions of the ingredients will vary, since differences in riboflavin content occur. The yeast used in this study was somewhat higher than most fresh yeasts which have been examined, since usually a value of about 20 μg per gram has been found. The nonfat milk solids also vary. The analyses of a large number of samples have given values ranging from a low of about 16 to a high of 25. The average was about 19 μg per gram.

Experimental

The microbiological method employed for the assay of riboflavin is essentially that described by Snell and Strong (1939). Preparation of the extracts for the assays was carried out by autoclaving 5- to 10-gram samples of the finely ground cereal with 90 ml of distilled water at 15 lbs pressure for 15 minutes. After cooling to about 50°C, 5 ml of 6% takadiastase solution was stirred in and the suspension allowed to stand for 1 hour. It was then made up to 100 ml with distilled water and clarified by centrifuging. All operations were carried out in a darkened room.

The takadiastase employed in preparing the extracts contained riboflavin. The amounts were, however, too small to significantly affect the analytical values. Preparations of takadiastase should be examined to insure that serious errors are not introduced from this source.

Summary

The riboflavin content of a variety of cereals and cereal products has been determined by the microbiological assay method. There is a close parallelism between the thiamin and riboflavin contents of wheat and its products of milling. No measurable loss of riboflavin occurs in the baking of bread but large losses are found when slices of bread are unduly exposed to light.

Acknowledgments

The authors are indebted to Mr. B. A. McClellan for preparing the samples of bread. The samples of cereal grains were kindly supplied by Dr. C. H. Bailey, University of Minnesota; Dr. John H. Parker, Kansas Wheat Improvement Association; Dr. B. H. Thomas, Iowa State College; and Dr. C. A. Lamb, Ohio Agricultural Station.

Literature Cited

- Andrews, J. S., Boyd, H. M., and Terry, D. E.
1941 Application of the microbiological method to the riboflavin analysis of cereals. Presented at the September, 1941, convention of the American Chemical Society at Atlantic City.
- Andrews, J. S., and Nordgren, R.
1941 Application of the thiochrome method to the thiamin analysis of cereals and cereal products. *Cereal Chem.* **18**: 686-695.
- Bourquin, Anne, and Sherman, H. C.
1931 Quantitative determination of vitamin G (B₂). *J. Am. Chem. Soc.* **53**: 3501-3505.
- Committee on Assay of Foods
1941 Vitamin B complex—the members of this group and status of methods of assay. *Am. J. Pub. Health Supplement* **31** (No. 3): 95-100.
- Conner, R. T., and Straub, G. J.
1941 Combined determination of riboflavin and thiamin in food products. *Ind. Eng. Chem. (Anal. Ed.)* **13**: 385-388.
- Ferrebee, J. W.
1940 The urinary excretion of riboflavin: fluorometric methods for its estimation. *J. Clin. Investigation* **19**: 251-256

- Hodson, A. Z., and Norris, L. C.
1939 A fluorometric method for determining the riboflavin content of food-stuffs. *J. Biol. Chem.* **131**: 621-630.
- Kammerer, A. R.
1940 Report on riboflavin. *J. Assoc. Offic. Agr. Chem.* **23**: 346-351.
- Norris, L. C., Wilgus, H. S., Jr., Ringrose, A. T., Heiman, V., and Heuser, G. F.
1936 The vitamin G requirement of poultry. *Cornell Agr. Exp. Sta. Bul.* **660**.
- Nordgren, R., and Andrews, J. S.
1941 The thiamin content of cereal grains. *Cereal Chem.* **18**: 802-811.
- Snell, E. E., and Strong, F. M.
1939 A microbiological assay for riboflavin *Ind. Eng. Chem. (Anal. Ed.)* **11**: 346-350.

ON THE MULTIPLE AMYLOSE CONCEPT OF STARCH.

II. AMYLOPECTIN AND AMYLOSE¹

RALPH W. KERR, O. R. TRUBELL, and G. M. SEVERSON

Research Laboratories, Corn Products Refining Co., Argo, Illinois

In a preceding paper (Kerr and Trubell, 1941) several of the more modern concepts favoring the view that starch is composed of only one type of molecule have been reviewed and shown to be untenable for corn starch. Therein it was demonstrated that there are at least two fractions, separate and distinct from each other, the one called simply amylose for the present, and the other gamma-amylose. In this paper it is proposed to show that the behavior of the starches is not explainable as consisting only of varying ratios of amylose and gamma-amylose, but that the starches are fabricated from more than two amyloses.

The bulk of the starch granule has been at various times during the last half century (and even up to the current year) conceived as divisible into two main constituents: amylose and amylopectin, or alpha- and beta-amylose, or fractions otherwise designated. The first question to be discussed is whether gamma-amylose is identical with the fraction frequently called amylopectin. A second issue is to consider the existence of amylopectin, itself, the presence of which as a chemical entity has recently been seriously questioned.

To introduce the discussion which follows, it is our desire to point out first that much of the confusion which has followed from apparently contradictory evidence in this field of work results largely from poor definition and from the promiscuous use of terms without due reference to the original concept for which these terms had definite meanings. For example, Taylor and Schoch (1933) deny the existence of amylopectin in potato starch, inasmuch as they could find no fraction of potato starch which corresponded to corn alpha-amylose, their equiva-

¹ Presented at the 101st meeting of the American Chemical Society, Division of Sugar Chemistry, St. Louis, Missouri, April 11, 1941.

lent of amylopectin. Moreover, they were unable to demonstrate that the fractionation of potato starch, by use of a cataphoresis cell, led to a separation of combined phosphorus in the component which migrates to the positive electrode. Samec (1927) had claimed such a separation and has characterized amylopectin as a phosphoric acid ester. From their work Taylor and Schoch conclude that potato starch is all soluble amylose—that is, contains no amylopectin. The errors involved in this logic are worth considering, inasmuch as many of the arguments denying the existence of hypothetical amylopectin in all starches follow an analogous pattern.

Taylor revived the terms "alpha"- and "beta"-amylose proposed by A. Meyer, stating that the differentiation rested primarily on solubility. Later Taylor and Iddles (1926) added to this concept their observation that the fatty acids of corn starch are concentrated in the alpha-amylose fraction. In the meantime Samec had developed his amylophosphoric acid theory for amylopectin. These investigators showed independently, moreover, that their hypothetical carbohydrate esters, alpha-amylose and amylopectin, migrated in a cataphoresis cell. From the evidence, Taylor must have considered alpha-amylose the equivalent of amylopectin, for Taylor and Schoch (1933) use the two terms synonymously.

In the first place Meyer himself was not satisfied with the concept of alpha- and beta-amylose, certainly not in using solubility as a prime basis of distinction, for in a historical review of the latter's work by Samec (1927) it is made evident that Meyer's prime basis of differentiation for alpha-amylose became finally its greater resistance both to diastatic enzymes and to acid.

The introduction of the concept of retrogradation by Maquenne at about this time was most opportune, for it gave a plausible explanation to the inconsistent results of Meyer, because of which results Meyer had become convinced that there was no fundamental difference between his two fractions. It was now apparent that most of Meyer's resistant amylose fractions contained retrograded amylose in varying amounts.

Unfortunately, however, Maquenne and Roux (1905) appear to have drawn the sweeping conclusion that all insoluble and resistant fractions of starch (amylocellulose, alpha-amylose, etc.) are but physical modifications of one substance, which was designated later simply as amylose. Nevertheless they were unwilling to concede that starch is one substance, chemically. To account for the fact that a part of starch is converted only as far as dextrin by diastase, whereas their preparations of amylose were believed to convert completely to maltose, the term amylopectin was introduced by these investigators (Maquenne

and Roux, 1905) to name the nonsaccharifiable portion. The choice of name was influenced by the fact that their saccharifiable fractions of starch did not gel, from which it was assumed that the fraction which formed dextrin was probably the same component which imparts to starch the property of paste formation. It should be clear, therefore, that this hypothetical amylopectin is not the equivalent of alpha-amylose. Indeed, the former has no counterpart in the alpha-beta-amylose nomenclature.

Not only Taylor, but many of his contemporaries who were ardent supporters of the multiple-amylose concept have inadvertently done as much as their opponents to cast suspicion on this concept, in the confusion which has resulted from the manner in which they have used these terms, with little consideration for the original concept, in designating their fractions.

Other workers, as for example Hanes (1937), appreciate fully the original distinctions drawn between amylose and amylopectin, but conclude that a differentiation on this basis is faulty, since it rests on a misconception of the mechanism of starch enzymolysis. Possibly it does. More will be said in this connection in our discussion which follows. Possibly, if starch is a nonhomogeneous mixture, some other basis for differentiating these fractions will be found to be the true one. But if we are to emerge from the confusion into which this field of work has drifted, it should be obvious that the revival of outmoded terms or the inaccurate use of other terms to describe various fractions of starch should first be discontinued.

Amylopectin as a Constituent of Starch

Tychowski (1937), accepting the modified procedures of Ling (1928) for estimating amylose, wherein barley diastase is used in place of malt diastase (which contains a factor strictly dextrinogenic and is incapable of producing maltose—Hanes, 1937), has more accurately determined the percentages of maltose formed from various starches as follows: potato, 63%; wheat, 65%; rye, 63%; barley, 62%; corn, 59%; and rice, 59%. From this and a further study (1937) he estimates that the amylose content of all starches is only approximately 58% of the total starch. The remainder is claimed to be amylopectin.

We have attempted first to determine more accurately this amylopectin value, while at the same time answering a fundamental objection to this method of analysis, and finally, to point out that gamma-amylose is not this hypothetical constituent. It has long been supposed that retrograded amylose is resistant to the diastases and it is not inconceivable that a part of the amylose as it exists in the starch granule is already in the retrograded form, in the sense that in ordinary

gelatinization procedures it is not sufficiently hydrated, dispersed, or otherwise conditioned to render it readily convertible. Thus, the failure of starch to be readily converted entirely to maltose might be explainable on a basis of preretrogradation of a part of the granule.

First of all, therefore, samples of corn, potato, and tapioca starches were thoroughly dispersed in 0.67*N* KOH, neutralized, and immediately converted with barley diastase in accordance with procedures given in the "Experimental" section. The results of this conversion are given in Table I.

TABLE I
DIASTATIC CONVERSION OF ALKALI GELATINIZED STARCHES

Conversion time <i>min</i>	Percent of dry substance starch converted to maltose		
	Corn	Tapioca	Potato
5	14.87	17.8	18.15
15	33.2	36.6	35.6
45	46.6	47.2	46.7
75	51.1	51.9	50.5
105	53.8	55.7	53.8
180	56.6	59.2	58.6
960	60.0	64.4	66.5

These values, when plotted graphically, show that with complete dispersion there still exists a definite change in the course of the reaction between 55% and 60% conversion. Up to this point the reaction curves for the three starches are very nearly superimposable, but beyond this point there is a secondary reaction which cannot be entirely explained, as has been attempted, by ascribing the continued rise in reducing value to traces of alpha-amylase in the enzyme preparation used—acting on a common substrate in each case, the dextrins from amylopectin. The course of this latter reaction is also influenced by the composition of the substrate, whether it is principally amylopectin dextrins or whether, as we intend to show, it is the latter plus gamma-amylose.

While our results in general agree with the experimental values of Tychowski, it is felt that a refinement in analyzing and interpreting these results would lead to a more accurate estimation of the actual amylose and amylopectin contents of these various starches by this general method.

First of all, in Figure 1 the logarithm of the percent starch converted into maltose is given against conversion time which shows strikingly the change from initial reaction rate to final. If these two reaction rates were extrapolated to their point of intersection, we might more

precisely locate the point at which the first reaction ceases and the second begins.

However, accepting the multiple-amylose concept, to be consistent one should presuppose that the reduction values shown in the first part of the curve are the sum of those from maltose, which originate from amylose through no intermediate stages, plus reducing values from higher products resulting from one or more independent reactions—the simultaneous degradation of amylopectin, gamma-amylose, etc.

With these assumptions the latter stage of the reaction should be in progress even during the initial reaction and the reducing values of

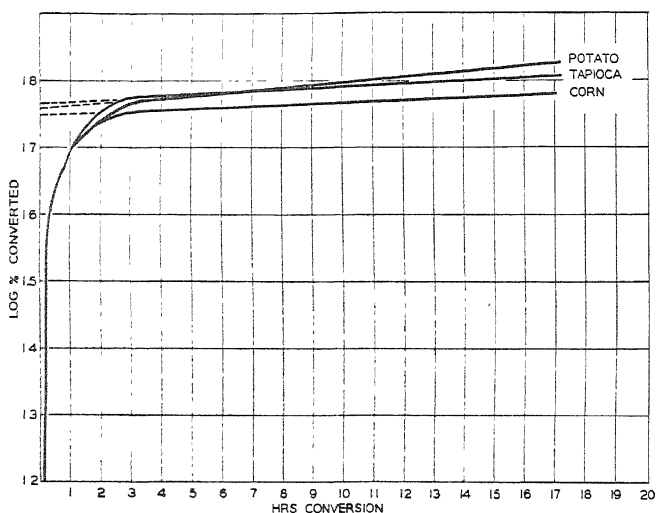


Fig. 1. Curves for potato, tapioca, and corn starch.

the products formed should be subtracted from the total found at completion of the first reaction in order to estimate the amount of amylose, only, converted. This deduction can be accomplished by extrapolating the logarithmic curve for the second reaction to zero time and noting the amount of maltose corresponding to this intersection point.

Figure 1 shows the logarithmic curves for corn, tapioca, and potato starch, from which we estimate that the amylose content of these starches are 55.8% for corn, 57.6% for potato, and 57.8% for tapioca.

We have previously shown (Kerr and Trubell, 1941) that tapioca and potato starches contain negligible quantities of gamma-amylose, less than 1% for tapioca and an amount for potato too small to isolate,

and we have estimated that corn starch contains approximately 10%. It should be evident, therefore, that the fraction, gamma-amylose, cannot account for all of that portion of starch which does not convert to maltose. There is a portion which in contrast to gamma-amylose is speedily converted to a more or less soluble, dextrinous material. This latter fraction, by definition amylopectin, should comprise then about 34% of the weight of corn starch, 41% of tapioca, and 42% of potato.

To show that dispersion in alkali is sufficient to condition retrograded amylose for conversion, the following experiment was attempted:

Tapioca starch was alkali-gelatinized as had been done previously and now neutralized to pH 4.5. It was allowed to retrograde under toluol for 10 days at 4°C. The entire mixture was then treated with barley diastase for 24 hours, in accordance with our procedures previously given (Kerr and Trubell, 1941) for preparing gamma-amylose. The insolubles were centrifuged and washed free of reducing substances in the centrifuge, and these resistant insolubles were now dissolved in 0.67*N* KOH at 25°C. After 30 minutes HCl was added to adjust the pH to 5.0 and after one hour's standing the gamma-amylose precipitating was centrifuged out. The centrifugate was immediately converted with barley diastase at pH 6.0 and 47°C; the maltose formed from time to time was determined by alkaline ferricyanide reduction and the percent of dry solids present converted to maltose was calculated (Table II).

TABLE II
CONVERSION OF RETROGRADED TAPIOCA STARCH

Conversion time	Dry substance converted to maltose
	%
15	41.5
30	59.4
75	70.8
150	72.9
210	73.1

It is apparent that while the conversion limit for this material is definitely higher than for whole starch, it is still incomplete, for when this conversion was plotted as was done for parent starch, a conversion limit of 71.6% was estimated. This latter result may be partly explainable on the basis that all of the more resistant gamma-amylose failed to precipitate from the solubilized retrograded amylose in one hour. However, whatever the explanation, it is apparent that retrograded amylose will convert with diastase after redispersion in

alkali and will do so at an accelerated rate, as compared with whole starch. It does not seem likely, therefore, that we can entirely explain the abrupt change in the course of the diastatic curve for conversion on a physical or physical-chemical basis, alone.

To settle the above question more conclusively, however, requires the clarification of many uncertainties. To obtain pure retrograded amylose presupposes that we obtain a pure amylose, procedures for which are still lacking. If we assume that amylose may be purified by the process of retrogradation, we limit ourselves to the proposition that only amylose retrogrades, in which case a better definition of retrogradation than the varied ones now prevalent is required.

A definition of retrogradation as the change of a soluble, non-resistant fraction of starch into an insoluble, more resistant form would include the precipitation from solution of corn gamma-amylose either by lowering the temperature or changing the pH of the solution. To exclude the above phenomena by limiting retrogradation to an irreversible change is not in accordance with the experiment above given. And to limit retrogradation to progressive precipitation from comparatively dilute solutions at lower temperature and normal acidities would ignore our experiments on gamma-amylose, in which it is seen that a distinction on this basis is only relative.

Amylose as a Constituent of Starch

Older methods of starch fractionation: The major problem before us, then, is the separation of amylose—that is, the separation of a fraction that will convert straightforwardly to maltose with barley diastase. In addition to the partially successful one, just given, by retrogradation, we have tried several of the more commonly used and quoted procedures purporting to give such a separation, but with unsatisfactory results. These are electrophoresis of a dispersed starch paste, simple hot-water extraction of starch granules, and freezing and thawing of starch pastes. In addition, we have evidence that a fifth method (Samec, 1929), precipitation with alkaline earths, would be unsatisfactory. Thus we have found that gamma-amylose also forms a difficultly soluble barium salt which is not readily fractionated by progressive solubilization from the mixture precipitated. This experiment will be treated in greater detail in a following communication.

Amylose by electrophoresis was prepared by procedures used by Taylor and his coworkers, but using autoclaved corn-starch pastes in accordance with procedures elaborated by T. J. Schoch. Hot-water-soluble amylose was prepared, as recently suggested by Meyer, Bretano, and Bernfeld (1940). However, our yield was only about one-third of the 16.0% obtained by Meyer in one hour's extraction at

80°C. Three independent extractions gave us yields of 5.4, 4.9, and 6.0% respectively.

Amylose by freezing and thawing was prepared by the well-known procedures used by Ling and by Pringsheim and Wolfssohn. A 5% paste of tapioca starch was frozen for 16 hours at -10°C. The mass was then washed with water at 60°C. Most of the slimy insoluble residue was thrown out by centrifuging and the centrifugate filtered. The soluble amylose yield was approximately 61% for tapioca starch. Each product was converted by procedures given for obtaining conversion data in Table I.

TABLE III
CONVERSION OF AMYLOSE FRACTIONS

Conversion time	Dry substance converted to maltose		
	Amylose by: Electro-phoresis	Hot water	Freezing and thawing
<i>min</i>	<i>%</i>	<i>%</i>	<i>%</i>
5	28.6	19.3	18.8
15	50.5	41.1	37.3
45	62.0	69.6	53.7
75	64.5	77.6	58.7
105	65.5	79.0	60.5
180	67.5	80.5	61.5
1200	71.0	85.0	66.9
Conversion limit	66.0	79.0	60.2

It is apparent from the above data, shown graphically in Figure 2, that only amylose by hot-water extraction has a significantly higher conversion limit than the parent starch. Amylose by cataphoresis has a measurably higher limit, but inasmuch as the paste for separation was made by autoclaving, this increase is perhaps understandable in view of the work cited by Hanes (1937, p. 198-200). The small yield of the amylose by hot-water extraction detracts from the significance of our results obtained and leaves these results open to the argument that we had extracted but a small quantity of amyloid material present in the granule in a state of incomplete synthesis to starch or material degraded from starch in the process of starch manufacture.

We do not agree with the last given interpretation for several reasons, but concede, however, that existing data do not support the view that approximately 60% of the weight of starch converted to maltose can be ascribed to the complete conversion of one distinct amylose. Conversely, then, it might appear that a more suitable interpretation would be that of Hanes, previously referred to, that the dextrins of beta-amylase conversion of starch are not the products from

a distinct component, amylopectin, but are, rather, the short-chain residues of a single and originally longer starch molecule. However, in examining this latter theory, the reason why the enzyme has greater difficulty in hydrolyzing the molecule as the chain becomes shorter is not satisfactorily explained. Moreover, since the concept of side chains or other enzymatic blocks has been subsequently introduced, the conclusion that these residues are necessarily of much lesser molecular magnitude has become even less convincing.

Great weight is attached by Hanes to end-group analysis by methylation, as indicative that these dextrans are 40% of the length of the

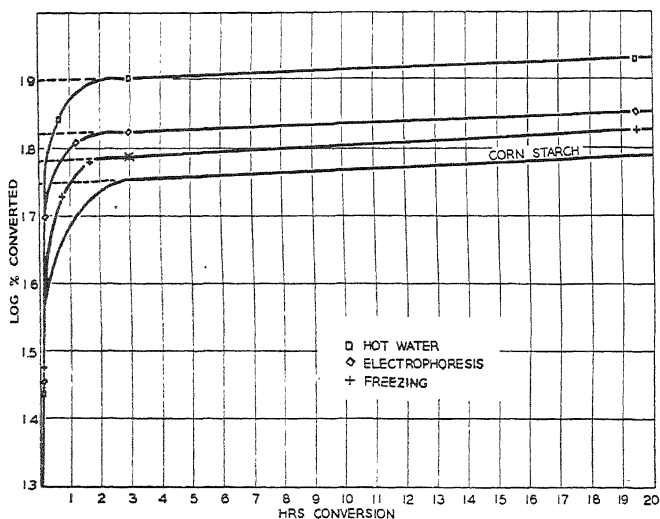


Fig. 2. Curves for amyloses by electrophoresis, hot water, and freezing.

starch molecule. However, even in arriving at an estimate of the original chain length of starch, Staudinger (1937) has pointed out that these results of end-group analysis may be interpreted differently. It is evident that it is possible to obtain the same distribution between tri- and tetra-methyl dextrose from a molecule many times larger than that given by Haworth, provided branching of the chain exists. And if starch possesses only one molecular configuration, as suggested by Hanes, it would be very difficult, except by supposing a side-branched structure, to account for the results of Freudenberg on the di-methyl products obtained from fully methylated starch (Freudenberg, 1940).

Moreover, the support given by Hanes to Haworth's concept appears to be less convincing, since Myrbäck (1938) has indicated by

other methods that these limit dextrans may approach the order of starch itself in molecular magnitude. While the absolute values of Myrbäck's results might be questioned, it appears probable that the values are relative.

With a reasonable doubt existing, therefore, as to the conclusions given by Hanes concerning Maquenne's hypothesis we attempted to fraction starch by two methods, given below, in order to shed further light on this problem.

Fractionation of starch by alcohol precipitation: Schoch (1941) has indicated that starch may be fractioned into dissimilar components by adding higher alcohols to autoclaved starch pastes and supercentrifuging the mixture.

To avoid the solubilizing effect of high temperatures on the gamma-amylase constituent and to avoid the criticism that prolonged autoclaving increases the convertibility of starch fractions, as claimed by Hanes, we used a homogenizer to effect dispergation of the starch; and to obtain greater ease in the precipitation of the pastes, we combined the use of butyl alcohol with the more soluble methyl alcohol. Our procedures, as detailed in the experimental section, give us two main fractions: one which is least soluble in alcohol-water mixtures and should contain nearly all of the gamma-amylase content of the starch plus noncarbohydrate residues and plus other amyloid material, and a fraction which is more soluble.

The last-named fraction is very interesting in that its alkali-labile number, determined according to Schoch and Jensen's (1940) modification of Taylor's procedures, is of a very low order, as low as the value for whole tapioca and potato starches, which themselves are only about one-half as alkali-labile as corn starch. Hence, to point out more clearly the possible chemical difference for constituents of this fraction, we determined and compared alkali numbers for the two fractions above mentioned, together with the alkali numbers for gamma-amylase, corn, tapioca, and potato starches. These values are given in Table IV.

TABLE IV
ALKALI NUMBER AND CONVERSION LIMIT OF STARCHES AND FRACTIONS

Starch, or fraction	Alkali No.	Yield	Conversion limit
		%	%
Alcohol, more soluble	5.7	55.6	56.6
Alcohol, less soluble	20.0	44.4	65.3
Corn starch (dioxane extracted)	11.7	—	59.4
Gamma-amylase	42.0	(11.55)	48.1
Alcohol, less soluble, less actual gamma-amylase content	12.7	32.8	71.0 (estimated)
Tapioca starch	4.7	—	—
Potato starch	7.8	—	—

Having demonstrated by alkali numbers that corn starch can be fractionated by alcohol precipitation, it would appear, from the following three considerations that there are at least three fractions in corn starch.

First, it was found that corn starch divided itself into 44.4% as less alcohol soluble and 55.6% as more alcohol soluble, and that the former fraction contained 26.1% gamma-amylose by our method of separation (Kerr and Trubell, 1941) or 11.55% of the weight of starch. This latter value agrees fairly well with our previous estimate that corn starch contains 10% gamma-amylose. Thus there is left 32.8% of the weight of starch, less soluble in alcohol, unaccounted for.

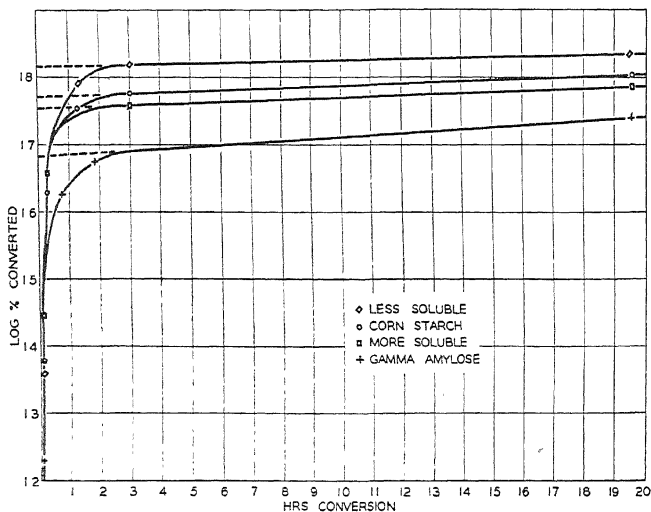


Fig. 3. Curves for conversions of various fractions.

Secondly, it is apparent that the alkali lability of corn starch cannot be accounted for as consisting of only gamma-amylose and the soluble fraction. For the alkali-labile number of the latter fraction is at least as low as 5.7, possibly lower if purified further, and assuming a composition of 1 part gamma-amylose to 9 of the soluble fraction, we arrive at a total value of only 9.3. It should be apparent then that there is room for a third fraction with an alkali number slightly higher than starch itself.

Lastly, to account for the alkali number of the less soluble alcohol fraction on the basis that it is only a mixture of one part gamma-amylose to three parts of the more soluble fraction involves an even wider discrepancy between found and estimated values, and points

more conclusively to the existence of a third component. Further differences in these fractions will now be pointed out. It will also be noted in passing, however, that the values obtained for the potato and tapioca starches leave little room for a component with as high an alkali number as gamma-amylose and we believe that this experiment explains, incidentally, the higher value for corn starch as compared to the two noncereal starches.

Diastatic analysis of starch fractions: The above-described fractions were converted with barley diastase in the manner already given and the conversions plotted in Figure 3, from which the limits of conversion given in Table IV were estimated. It will be observed that

1. The soluble fraction converts to a slightly lower limit than whole starch. This material, as a whole, then, is not the hypothetical amylose of Maquenne.

2. The initial conversion rate extends further with the less soluble fraction than with starch or the other two fractions of starch.

3. Gamma-amylose, solubilized by alkali before conversion, reaches a conversion limit much sooner than the other fractions.

Knowing that the less soluble fraction is one-fourth gamma-amylose, it is apparent then that the remaining part of this fraction has a conversion limit of over 70%. It is possible, therefore, that were this portion freed of gamma-amylose and of impurities, probably of the more soluble type, a relatively high conversion limit would be found for this fraction, even of the order of that shown for Kurt Meyer's hot-water-extracted amylose.

It is significant, we believe, that our most readily converted fraction should be found in that part that is least soluble, whereas Meyer's amylose is apparently very soluble. It is our opinion that solubility is, therefore, a poor general criterion for amylose and the indications are that amylose may be a series of products related chemically but with varied physical properties. It is understandable then how one might be led to conclude as did Ling, that "amylose" would never be completely and quantitatively separated because only a part of it, as he expressed it, is in the "free" condition.

Separations by fractional precipitation of starch acetate: Although it is apparent that dissimilar fractions are obtainable from corn starch by fractionally precipitating a paste with alcohols, as judged by the diastase convertibility and the alkali labile number of these fractions, purification of these fractions appears to be difficult because precipitation is not clean cut. The precipitates are thrown out with great difficulty so that even after reprecipitation of these fractions one is not sure that considerable adsorption of the more soluble fraction on the less soluble has been avoided, and it is almost certain that a small

portion of the less soluble fraction remains colloiddally suspended in the more soluble fractions.

To overcome these difficulties the fractional precipitation of starch triacetate was attempted by gradual additions of petroleum ether to solutions in chloroform in more or less the same manner that the acetates of the limit dextrans have been fractioned in our laboratory.

Both the preferred method for acetylation of corn starch, developed for this particular work, and the method of fractionation of the acetates are given in the experimental section.

Three distinct fractions were prepared. One was a fraction which swelled in chloroform to a gel-like nature and did not truly dissolve. This would correspond in superficial properties, at least, to an acetate fraction described by Hanes (1937, p. 124), purported to be an amylopectin acetate. A second fraction was obtained, soluble in chloroform

TABLE V
FRACTIONS OF CORN STARCH TRIACETATES

	Yield	Acetyl	Beta-amylase conversion limit
	%	%	%
Least soluble fraction	34.4	41.9	57.5
Intermediately soluble fraction	52.25	43.0	75.2
Most soluble fraction	13.25	45.7	69.9
Parent starch triacetate	—	43.5	63.1

with a rather high viscosity therein but insoluble in mixtures of chloroform containing higher than 57.2% by volume of petroleum ether. It precipitated as a flocculent mass. A third fraction was obtained, soluble in chloroform-petroleum ether with a concentration of the latter higher than 57.2%. It precipitated over a broad range, but very nearly completely, however, at about 70% petroleum ether concentration. It deposited first as a gummy material which changed over to a refractive, granular powder when treated with petroleum ether, whereas treatment of the other two products with petroleum ether resembled the dehydration of a concentrated water solution of a dextrin with alcohol. Freed of their residual chloroform by washing with petroleum ether, they were dried with the following yields, as shown in Table V.

Acetyl groups were determined by the method of Murray, Staud, and Gray (1931) using pyridine as a dispersing agent, but this procedure did not provide a completely homogeneous reaction mixture, either for the starch triacetate or for the chloroform insoluble fraction. This is one factor, possibly, that accounts for the analyses showing values

less than the theoretical 44.8% acetyl. Another factor may have been the use of the whole starch triacetate, which contains approximately 1% noncarbohydrate impurities such as adsorbed or combined ash, protein, and lipid material not readily extractable by common organic solvents. These impurities might accumulate in the least soluble fraction and proportionally decrease the apparent acetyl value for this fraction.

Larger quantities of these fractions, together with the parent starch triacetate, were all deacetylated in the same manner, and after neutralization of the deacetylated reaction mixtures the regenerated

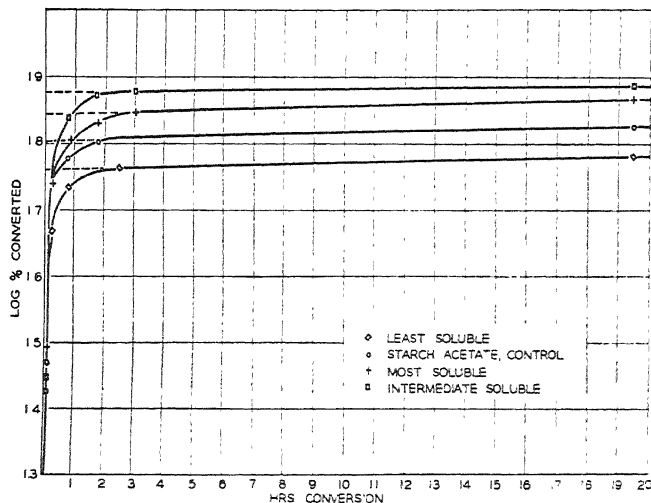


Fig. 4. Curves for conversions of various fractions.

carbohydrate fractions were precipitated by adding enough ethyl alcohol to result in a 50% (by volume) alcohol solution. After centrifuging, the precipitates were washed free of pyridine with alcohol, then with ether, and finally dried *in vacuo*. These products were completely dispersed in 0.67*N* NaOH, neutralized to pH 5.0 with HCl and converted with barley diastase by procedures identical to those used on the starches and other starch fractions (see experimental section).

The results of these diastatic conversions are given in Figure 4, from which the conversion limits shown in Table V have been estimated. It is apparent that even greater differences in convertibility result with these fractions than with starch paste fractionated by alcohol precipitation. It is to be noted that the fraction intermediately soluble in

respect to the acetate derivative, is now by far the most readily converted.

It is our belief that refractionation of this portion will yield components showing an even higher limit of conversion. Indeed, it is to be expected that each of the two more soluble fractions may in turn be further subdivided into their constituents by an extension or elaboration of the fractionation given.

With the theory of starch composition apparently becoming more complex, it would seem, therefore, that the major problem before us is the isolation in pure form of these several components, for which a new terminology seems to be very much needed. While Maquenne's amylose-amylopectin theory, which superseded the alpha-beta-amylose concept, has not as yet been shown to be an impossible one, it must be admitted that the theory is still highly speculative and possibly an oversimplification of the composition of the starches.

Experimental

Conversion of starch products with barley diastase: Two grams of product (dry basis) were wet with 10 ml of water to which was then added, with stirring, 20 ml of normal NaOH. Stirring was continued for 30 minutes at 25°C. One hundred ml of water was then added and the solution was carefully adjusted to pH 5.8 with HCl, using the glass electrode. The volume was then brought immediately to 250 ml, readjusting the pH, if necessary, and 2 ml of barley diastase (the extract equivalent to 0.5 g of barley) added as soon as possible after bringing the starch product solution to 47°C.

Aliquot portions were removed at definite intervals, pipetting directly into a mixture of sodium carbonate and ferricyanide and the maltose estimated according to the method of Gore and Steele (1935). The percentage of starch converted to maltose was then calculated after deduction of the value for a blank made by pipetting proportionate amounts of both enzyme and starch solution into the alkaline ferricyanide. The preparation of our barley diastase has been previously described (Kerr and Trubell, 1941).

Separation of starch components with alcohols: A 3% suspension of dioxane-extracted corn starch, at pH 5.5, was brought up slowly to 90°C, held for 30 minutes, and then cooled quickly to 65°C, whereupon it was immediately run through a two-stage homogenizer (Viscolizer, Junior) at 4,300 lbs of pressure. Under these conditions, dispergation of the starch appeared to be complete. The liquors emerging at 55°C were immediately saturated with normal butanol and allowed to stand overnight. Methyl alcohol was now added slowly with vigorous agitation until the content of the latter was 15% by volume and stirring

was continued 3 to 4 hours. The insolubles resulting were then centrifuged out and washed once in the centrifuge with a mixture containing approximately 75% water, 10% butanol, and 15% methanol.

The insolubles were further purified by resuspension in about 85% of the water used to make up the original starch paste and brought up to a boil slowly with very thorough stirring. As the paste cooled it was again saturated with butyl alcohol and after an hour or so at room temperature, methyl alcohol was again added to a content of 15% by volume, and allowed to stand overnight. Thereupon the mixture was centrifuged, dehydrated with successive washings in methyl alcohol, and finally dried over H_2SO_4 in a vacuum desiccator.

The three centrifugates were separately treated. The solubles in each were precipitated by adding an excess of methyl alcohol, settling overnight, decanting, and taking up the precipitate in methyl alcohol.

The yield of the four products was distributed as follows:

As less soluble	44.6%	
As more soluble	55.6	
a. From first centrifugate		47.4%
b. From wash of insolubles		7.8
c. From reprecipitation		0.4

For the tests described in the text, only the soluble fraction (a) was used, the others (b and c) appearing to be less pure, particularly the (c) fraction.

Preparation of whole starch triacetate: Powdered corn starch was carefully dried to between 2% to 3% moisture. To each gram of starch in a flask with agitator and condenser, 0.12 g of fused sodium acetate and 3.3 g of cp acetic anhydride were added quickly and the mixture stirred under anhydrous conditions. Heat was gradually applied and the mixture refluxed for 4 days with continued agitation until the mixture became more or less homogeneous. However, even with prolonged refluxing using larger quantities of acetic anhydride the solution never became perfectly clear.

The mixture was then run slowly into a large volume of water with vigorous agitation, regulating the pH, if necessary, from time to time within the range of 4.5 to 5.0. After several washings of the product, it was pulverized, washing was continued until the washings were substantially free from acetate ion, and it was then air dried. Acetyl found: 43.5%.

Separation of starch components by fractionally precipitating them as acetates: Twenty grams of whole starch triacetate were suspended in 600 ml of chloroform in a closed vessel and allowed to stand with intermittent stirring for about 10 days. Thereupon, 600 ml of petroleum ether was gradually stirred in and the mixture centrifuged in closed bottles. The centrifugate was decanted and the residue was

washed by suspending in a mixture of 100 ml of chloroform plus 100 ml of petroleum ether and recentrifuging. The residue was further purified by stirring up with 400 ml of chloroform, adding 300 ml of petroleum ether and centrifuging. The residue was then washed with petroleum ether until substantially free from chloroform and then dried.

The washings from the above were evaporated and added to the first centrifugate to which was then added with constant agitation 200 ml more petroleum ether and the precipitate was centrifuged out. The insolubles were washed with a small amount of a chloroform-petroleum ether solution (3 : 4), then with petroleum ether until chloroform free, and then dried. To the centrifugate an addition of 300 ml more petroleum ether brought down substantially the balance of the original acetate, which was washed with petroleum ether in the centrifuge and then dried.

Conclusions

Refinements in procedures are given for the estimation of amylopectin in corn, tapioca, and potato starches, from which it is shown that the percentages calculated from diastatic conversion are not as constant for these three types of starch as has been reported by earlier investigators.

The material not saccharified by diastase cannot be accounted for solely as gamma-amylose, from which it is concluded that the latter is not identical with amylopectin.

The more common procedures for separating amylose are reviewed, but in no case was a product obtained which converted straightforwardly and quantitatively to maltose with beta-amylase.

The products from two newer methods of fractionating starch are reported but none of these fractions answers the requirements for either amylopectin or amylose.

The concept of amylopectin and amylose, given by Maquenne, is still apparently hypothetical, and until it can be demonstrated, caution is advised in the use of these terms for designating various fractions of starch.

Literature Cited

- Freudenberg, K., and Boppel, H.
1940 The position at which branching occurs in the starch chain. *Naturwissenschaften* 28: 264.
- Gore, H. C., and Steele, H. K.
1935 Estimation of the saccharifying power of malt diastase. *Ind. Eng. Chem. (Anal. Ed.)* 7: 324-326.
- Hanes, C. S.
1937 The action of amylases in relation to the structure of starch. *New Phytologist* 36: 101, 189-240.
- Kerr, R. W., and Trubell, O. R.
1941 On the multiple amylose concept of starch. I. Gamma-amylose. *Cereal Chem.* 18: 530-548.

- Ling, A. R.
 - 1928 Enzymic hydrolysis of starch in relation to constitution. Survey of Starch Chemistry, Walton. Chemical Catalog Company, New York, p. 24.
- Maquenne, L., and Roux, E.
 - 1905 Sur la constitution, la saccharification et la rétrogradation des ampois de Féculé. *Compt. rend.* **140**: 1363-1368.
- Meyer, K., Bretano, W., and Bertoldi, P.
 - 1940 Studies on starch. *Helv. Chim. Acta.* **23**: 845-853.
- Murray, T. F., Staud, C. J., and Gray, H. LeB.
 - 1931 Acid value of cellulose fatty acid esters and rapid analysis of certain cellulose acetates. *Ind. Eng. Chem. (Anal. Ed.)* **3**: 269-273.
- Myrback, K.
 - 1938 Limit dextrin and starch. *Biochem. Z.* **297**: 164-178.
- Samec, M.
 - 1927 Kolloidchemie der Starke. Leipzig.
 - 1929 Potato amylopectin prepared and studied. *Lebensmittelchemie. Biochem. Z.* **205**: 104-114.
- Schoch, T. J.
 - 1941 Physical aspects of starch behavior. *Cereal Chem.* **18**: 141-148.
- and Jensen, C. C.
 - 1940 A simplified alkali-stability determination for starch products. *Ind. Eng. Chem. Anal.* **12**: 531-532.
- Staudinger, H.
 - 1937 Über Cellulose, Stärke und Glykogen. *Naturwissenschaften* **25**: 675-681.
- Taylor, T. C., and Hillis, H. A.
 - 1926 Separation of the amyloses in some starches. *Ind. Eng. Chem.* **18**: 713-717.
- Taylor, T. C., and Schoch, T. J.
 - 1933 Potato starch. *J. Am. Chem. Soc.* **55**: 4248-4255.
- Tychowski, A. S.
 - 1937 Splitting of starches of different origins by different cell amylases. *Biochem. Z.* **291**: 133-153, 247-253.

QUANTITATIVE CONTROL TEST FOR IRON ADDED TO FLOUR

J. T. FLOHIL and R. O. BROWN

Pillsbury Research Laboratory, Minneapolis, Minnesota

Read at the Annual Meeting, May 1941

The control of the addition of iron to flour is important because it, in turn, implies control of the distribution of concentrate and added vitamin in enriched flour. The following method for the estimation of added iron in enriched flour is recommended for control work, where an accuracy within 0.5 to 1 ppm is considered satisfactory. Its application is limited to ferric iron compounds soluble in dilute HCl acid. The test is based on a colorimetric procedure, in which use is made of a series of five carefully prepared standards. Its accuracy depends on certain limits of added iron. For this reason the conditions of the tests have been chosen so as to conform to the prevailing practice, which calls for the addition of approximately 10 ppm. In the preparation of the standards, two distinct methods may be employed.

First Method

Prepare a series of flour standards using, as a base, flour of the same milling grade and mixture as the sample or samples to be tested and providing the following five stock standards: 120%, 110%, 100%, 90%, and 80% of the regular iron treatment.

These standard samples are most conveniently made by first preparing 2,000 g of flour containing 120% of the normal iron treatment. Then, by dilution of 400-g portions of this flour with the calculated amount of untreated flour, the other four flour standards can be obtained.

Apparatus

2 dozen small test tubes 4" \times $\frac{1}{2}$ "
50-ml Griffin beakers
10-ml pipettes
2-ml pipettes
1-ml pipette in 0.1-ml divisions
Stirring rods

Reagents

Dilute HCl: 1,000 ml concentrated HCl (sp gr 1.18) diluted with 1500 ml distilled water.

Potassium ferrocyanide solution: 20 g potassium ferrocyanide in 100 ml distilled water.

Procedure: Four g of the five standard samples, respectively, are transferred to the 50-ml beakers, also 4-g portions of the sample or samples which are to be tested. Then to each beaker 10 ml of distilled water is added, and the flour and water are stirred together until a smooth suspension, entirely free of lumps, is obtained. To these suspensions, 10 ml of dilute HCl acid is added, and the contents of the beakers are properly stirred, left to stand for a few minutes, and then stirred again. After standing for about 45 minutes, enough supernatant liquid is available for the withdrawal of 2-ml portions.

These 2-ml portions are transferred to test tubes to each of which 0.1 ml of the potassium ferrocyanide solution has already been added. The tubes are shaken and then left to stand for about 30 minutes, when the color comparison of the unknown sample or samples with the standards may be made. If a centrifuge is available, which employs 50-ml tubes, much time can be saved by transferring the 4-g flour samples directly to the centrifuge tubes instead of the beakers. Centrifuging the suspension will naturally produce a much quicker and more complete separation of the liquid and solid phases of the suspension.

Second Method

The apparatus is the same as for the first method with the addition of a 2,000-ml volumetric flask, and the procedure is a modification of

the first method. Instead of a series of five flour standards, five stock samples of iron solutions in dilute acid are kept for reference standards. This has the advantage that it avoids a possible mixing error in the preparation of the standard flour samples.

These standard iron solutions are made up as follows: Calculate the weight of the iron compound to be dissolved in 2,000 ml of dilute acid on the following basis: 10 ml of this solution must contain the equivalent of 120% of the intended amount of added iron in 4 g of the enriched flour. Transfer this calculated amount of iron compound to a 2,000-ml flask, then add 800 ml of concentrated HCl acid, fill to the 2,000-ml mark with distilled water, let cool to room temperature, again fill to mark, and finally mix thoroughly.

Measure exactly 400 ml of this solution and transfer to a 500-ml Erlenmeyer flask. This portion is set aside as 120%. Again measure exactly 400 ml, transfer to a 500-ml Erlenmeyer flask, and add 36.5 ml of dilute HCl (1 to 1.5). Thoroughly mix. This is set aside as 110%. Again, 400 ml is measured and transferred to a 500-ml flask. To this 80 ml of dilute acid is added, thoroughly mixed, and set aside as 100%. The next 400 ml is transferred to a flask or beaker of a capacity considerably larger than 500 ml, and 133.3 ml of dilute acid is added and thoroughly mixed. This is the 90% standard. The fifth and last 400 ml is diluted and mixed with 200 ml of dilute HCl. This is the 80% standard.

It is evident that these standards must be kept free from evaporation or contamination.

Method of procedure: Weigh five 4-g portions of a flour (not enriched) of the same milling grade and mixture as the sample or samples to be tested for added iron. Transfer to beakers as above; or if a centrifuge is at hand, transfer directly to the 50-ml centrifuge tubes. The same procedure is followed with the 4-g portion, or portions, of the enriched samples to be tested.

To each of the beakers or centrifuge tubes, 10 ml of water is added; flour and water are thoroughly stirred with a stirring rod until smooth. Add to the five suspensions, representing the unenriched flour, 10 ml of the five standard iron solutions—120%, 110%, 100%, 90%, and 80% respectively. To the suspensions of the enriched sample or samples which are to be tested, 10 ml of dilute acid is added. They are stirred, left to stand for a few minutes, and then stirred again, after which they can be either centrifuged or left to settle out for about 45 minutes. Two-ml portions of the supernatant liquid are transferred to the small test tubes, to each of which has previously been added 0.1 ml of potassium ferrocyanide solution. The tubes are thoroughly shaken, to insure complete mixing, and are then set aside for about

half an hour, after which color comparisons can be made. It is best to make comparisons with reflected light.

Remarks

As already mentioned, the sensitivity of the test depends on certain limits of iron concentration. Under the conditions of the present methods of procedure, it was found that a 4-g flour sample is well adapted for prevailing conditions of iron fortification (approximately 10 ppm). If, at some future date, the present level should be changed, it will be advisable to change the size of the flour sample accordingly. If, for instance, the iron dosage should be doubled, only 2 g instead of 4 g must be used as the flour sample in this method.

A convenient way to clean the test tubes is to rinse them with concentrated KOH solution, then with water, followed by dilute HCl, tapwater, and finally, distilled water.

It is desirable that the test tubes used for this colorimetric procedure be carefully matched as to uniformity.

The change of the base flour at any change in the milling mixture involves the preparation of new standards in the case of the first method, but not in the second method. The second method therefore deserves preference.

The change of the base flour at every change in the milling mixture is necessary because of the variation in the ferric iron content of different wheats and their milling products.

THE RELATION OF FATS TO TEXTURE, CRUMB, AND VOLUME OF BREAD

J. C. BAKER and M. D. MIZE

Wallace & Tiernan Laboratories, Newark, New Jersey

(Read at the Annual Meeting, May 1941)

The results accomplished by the use of fats in bread making are a change in flavor, generally considered desirable; a more tender crumb; and in most instances a noticeable improvement in the quality of the bread when judged by texture and loaf volume. Comparatively little research on the action of shortening in bread making has been recorded during the past twenty years. Platt and Fleming (1923) reviewed and interpreted the action of shortening in bread making. Fisher and Jones (1932) studied and described effects of various shortenings. Heald (1937) reported the effects of varied amounts of several commercial shortenings. Bohn and Bailey (1937) measured the action of

shortenings on the physical properties of bread doughs. Sullivan (1940) studied and recorded the effects of substituting various shortenings for the natural flour fats in extracted flour. It is the purpose of this discussion to consider only the effects of fats upon the texture and loaf volume of the finished bread.

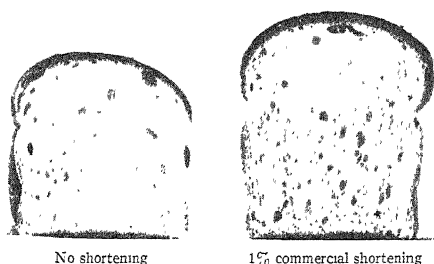


Fig. 1. Effects of shortening on bread.

Figure 1 illustrates the effects produced by 1% of commercial semi-solid shortening when used in dough that is matured to its optimal baking point with sodium chlorite and baked with the no-time method. The main difference produced by the use of the shortening is marked increase in volume with somewhat more open texture. This type of result is typical of the effects of shortening, though the no-time method of baking intensifies the differences. Heald (1937) found that all classes of shortening, 2% to 4%, increased the loaf volume of the bread.

In this paper the no-time method described by Baker and Mize (1941) has been used throughout. However, the technique there described has been changed by using a commercial molder in place of an extruder. To obtain the best results it is necessary to mold such doughs immediately at the end of the mixing period. This eliminates many variables that are present in other methods of baking and thus simplifies the interpretation of results.

Figure 2 shows no-time bread in which 3% of fat is used in liquid and also semisolid form. The increased amount of semisolid fat has produced an improvement in texture over that obtained by the 1%. However, when the liquid fat was used in this dough some loss in volume and noticeable deterioration of texture occurred. Apparently there is an effect caused by the physical state of the fat. This difference produced by fats was noted by us in 1939 and is also observed in baking straight 2½-hour doughs. Results similar to those produced by liquid fat were obtained with many liquid shortenings such as salad oil, cottonseed oil and peanut oil. Results comparable to those with the

semisolid fats have been obtained with all other commercial shortenings such as lard, cocoa butter, tallow, commercial hydrogenated shortening, etc. An improvement was invariably obtained in the quality of bread baked when such semisolid fats were used.

These observations led us to test the effect of hard, brittle fats in bread making. Such nonplastic fats are not available in nature but by complete hydrogenation fats can be made so hard they can readily

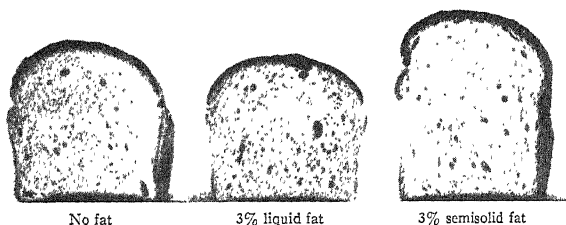


Fig. 2. Typical effects of liquid and semisolid fats on no-time bread.

be ground in a mortar. Completely hydrogenated cottonseed oil was ground and sieved to several mesh sizes up to 100 mesh and mixed in the doughs during the entire mixing period. The finer the mesh used the better the result obtained.

Usually greater improvement in the quality of bread was obtained by the use of this brittle, hard fat than with commercial shortenings. It gave increased volume and finer texture, as shown in Figure 3.

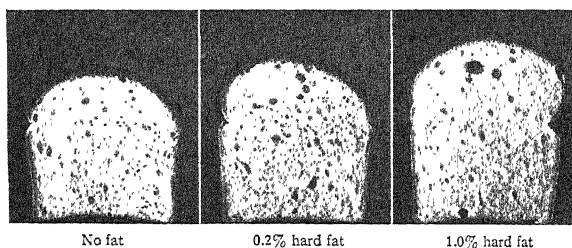


Fig. 3. Effects of hard fat on no-time doughs.

It is apparent that 0.2% has given some improvement and 1% has given marked volume and texture improvement. Comparing the effect of 1% of hard fat in Figure 3 with 1% of commercial shortening in Figure 1 it is noticeable that the hard fat has produced better results. Other hard substances such as carnauba wax, hard Russian paraffin, and Chinese insect wax, were ground to fine powder and tested in a

manner similar to that shown above. These materials gave an improvement in bread volume and texture similar to that of the completely hydrogenated cottonseed oil.

Figure 4 shows the results obtained by dissolving a small amount of hard fat in a liquid fat. Two percent of completely hydrogenated fat was dissolved in linseed oil. This was more than the minimum required to give a cloudy appearance in the oil. The total amount of hard fat added here amounts to only 0.06% calculated on the amount of flour used in the dough. Apparently this small amount of hard fat dissolved in the liquid fat exceeded its solubility when cooled, as shown by the cloudiness, and thus, due to fineness of the fat crystal, was able

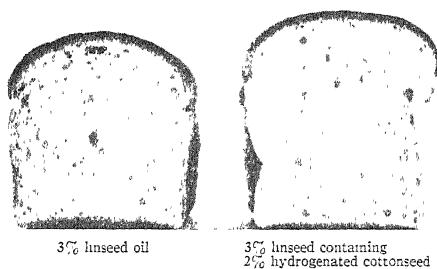


Fig. 4. Effects of solid fat blended with liquid fat.

to produce a noticeable effect with a smaller amount of hard fat than in any of our other experiments.

It is apparent that the improving action of such hard powders cannot be due to the often cited lubricating property of shortenings. A common explanation of the improving action of shortening is that it lubricates the fibers or filaments in the dough so that they slip by each other and better cell structure is thereby obtained. Certainly this explanation is not applicable to the results obtained from these hard powders. Neither is it satisfactory in explaining the results obtained by either semisolid fats or the fluid fats, for were the explanation true fluid fats should give results approaching those of semisolid fats since their lubricating properties are very pronounced.

Cocoonut oil has a sharp melting point slightly below the temperature at which doughs are ordinarily handled. This enabled us to test the effect of the physical state of a fat on doughs by mixing and proofing at temperatures above and below the melting point of the fat. The cocoonut oil used here had a melting point of 78°F and a solidifying point of 71°F. We therefore mixed two series of doughs, one at 80°F and the other at 70°F, and maintained these temperatures for each dough throughout the mixing and proofing period.

Figure 5 gives the result obtained from this experiment. These results show that when the bread was mixed and proofed at 70°F it made little difference whether cocoanut oil was added to the dough as a solid or a liquid. Substantially the same improvement was obtained. In contrast to this, cottonseed oil when mixed at this temperature gave a very poor loaf. However, when doughs were mixed at 80°F no improvement in volume was obtained by the use of cocoanut oil in either solid or liquid form though the solid form gave some improvement in texture. This was doubtless due to the small difference between the melting point of this fat and the temperature of mixing so that a

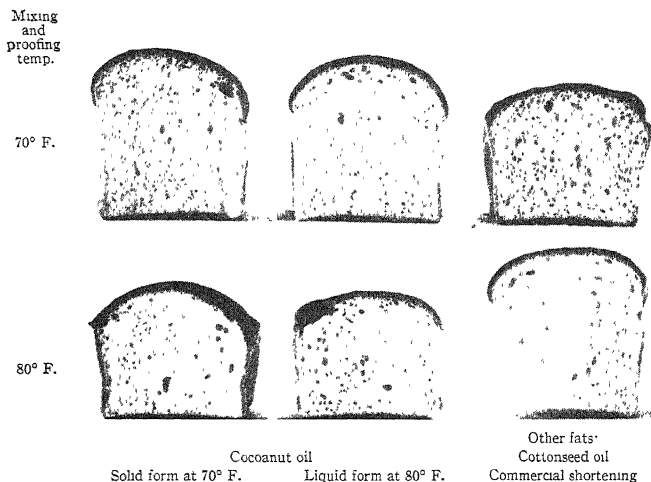


Fig. 5. Relation of dough temperature to effect of cocoanut oil in bread making.

considerable period may have elapsed before the solid fat became liquefied during the mixing, thus producing some of the effects of a solid fat. However, when cocoanut oil was added in a liquid form results very similar to that with cottonseed oil shown above were obtained. Commercial shortening was used at this higher temperature, showing that semisolid fat which does not liquefy at 80°F. produced good results at this temperature. This experiment proves that the effects obtained from a given fat are largely determined by whether it is a solid or a liquid at the temperature of the mixing and proofing.

In an attempt to find out something about the changes that occur in fat when mixed with dough, gluten was washed from such dough at the completion of the mixing period, before panning. Such doughs were carefully washed in 3% sodium chloride at the same temperature

as the dough at the end of the mixing. Thus no change in the salt balance of the ingredients was encountered during the washing.

In all cases where liquid fats were employed very little fat was recovered during the washing process. Substantially all the fat remained in the gluten except a very small amount which was held in colloidal suspension in the wash water. By centrifuging the wash water this fat could be separated.

When doughs made with semisolid fats are washed, the gluten retains the major portion of the fat. However, a considerable amount separates and floats on the wash water and can be skimmed off and collected. When solid fats are used, similarly a considerable portion remains in the gluten but another considerable portion floats on the wash water in its original condition and can be recovered.

TABLE I
EFFECT OF TEMPERATURE ON THE BLENDING OF COCOANUT FAT
WITH FLOUR FAT DURING MIXING
Three percent (10.2 g) coconut fat used per loaf.

Mixing temperature	Washing temperature	Amount fat recovered	Iodine number
85°F	65°F	0.156 g	26.0
65°F	65°F	5.6 g	15.2
<i>Theoretical Calculations</i>			
10.2 g coconut fat		I ₂ No. 9.5	
3.4 g flour fat		I ₂ No. 110.0	
Blended 13.6 g mixed fat		I ₂ No. 34.6	

Table I shows the effect of temperature on the blending of coconut fat and flour fat during dough mixing. Two doughs were mixed at different temperatures, one above the melting point of coconut oil and the other below. After the mixing, the warmer dough was cooled to the temperature of the cooler one. The gluten was then washed at the lower temperature from each dough. The weight and iodine number of the fat skimmed from the surface of the gluten wash solutions were determined.

Three percent (10.2 g) of coconut fat was used; the flour contained 1% (3.4 g) of natural fat. If all of the fat in the dough had been recovered on the surface of the gluten wash solution, 13.6 g of fat would have been obtained. When the dough was mixed above the melting point of the coconut fat very little was recovered, indicating that the fluid oil had completely blended with the dough and was all retained in the gluten with the flour fat. One might assume that the coconut fat and the flour fat had blended at this temperature of mixing. That this was the case is indicated by the high iodine number of 26 in the small

amount of fat recovered. Coconut oil has an iodine number of 9.5. A mixture should have a theoretical iodine number of 34.6. When the dough was mixed below the melting point of the coconut oil, at 65°F. incomplete blending of the fats was indicated by the iodine number of 15.2 and by the large amount of fat which floated on the gluten wash water. These figures indicate that a considerable proportion of the semisolid fat blended with the flour fat as shown by the iodine number of that portion recovered. A much larger proportion has been retained in the gluten and doubtless is still more closely blended with the flour fat.

These experiments suggest that the effects, which account for the improvement in bread obtained by the use of fats, are due to the blending of the added fat with the natural oil of the flour, thus producing changes in the properties of this natural oil. Most liquid fats, though they blend completely, are incapable of raising the melting point or increasing the viscosity of the flour fats. They blend together but still remain fluid. Semisolid fats, on the other hand, are capable of blending with the flour fat and raising its melting point and increasing its viscosity.

Castor oil acts differently from other liquid fats. Fine improvement of bread quality is obtained by its use, giving results very similar to the effects of commercial shortening.¹ This oil has a very high viscosity which does not drop as rapidly with increasing temperatures as does the viscosity of other oils. These characteristics suggest that castor oil operates in the same manner as other fats that improve bread quality—namely, by changing the character of the natural fats in the bread film, which by their fluid properties when heated permit gases to pass through the films. It may be that the highly viscous castor oil itself plugs these openings, thereby preventing porosity.

In a previous article (1939, p. 693) we published a chart that shows clearly the effects of shortening on optimum oxidized dough. In the test penetrability, pressure, and volume of dough while being baked in the electric oven were correlated and show that all three doughs, *i.e.* with no fat, with liquid fat, and with semisolid fat, have the same penetrable characteristics. Only the dough containing the semisolid fat shows the characteristic increase in pressure which accompanies increasing loaf volume, as contrasted to the others containing liquid fat or no fat. At that time we said "One is led to speculate as to why this should be. The fine texture of all of these loaves shows that there was no coalescence, nor was the cell structure disrupted as was shown by their final ability to retain gas. Apparently gas must escape from these doughs during the softening period by diffusion through the cell

¹ E. A. Fisher and C. R. Jones in 1932 observed this improving action of castor oil.

walls; hence the difference in their ability to retain gas may be a difference of cell-wall porosity. Here is a clew: solid [semisolid or hard] shortening may prevent cell-wall porosity; whereas fluid shortening or no shortening is unable to do so." We believe this observation is further proved by our present work.

In a paper by Baker (1939) a statement was made (p. 732) that semi-solid shortenings increase the permeability of bread by air. Furthermore, as stated on page 733, "Breads from some small bakeries are usually highly permeable, excepting those made without the use of shortening, in which case they are generally fairly impermeable even when of very coarse texture. Apparently shortening has an effect which prevents the cellular structure from finally closing or sealing the pores. This is directly opposite to the action of shortening during the earlier stages of baking, as shown in a previous paper."

TABLE II
EFFECTS OF FATS ON AIR PERMEABILITY OF BREAD
Matured no-time dough.

	Permeability	Volume <i>ml</i>	Texture
3% commercial shortening	43	2,640	102
1% commercial shortening	34	2,470	102
1% completely hydrogenated cottonseed	34	2,440	103
3% cottonseed oil	11	1,970	100
No fat	9	2,060	103
3% mineral oil	48	1,800	97

We have further investigated this permeable property of bread in connection with certain shortenings as shown in Table II. It has been noted that commercial shortenings and hard, completely hydrogenated fat produce bread of high permeability, whereas cottonseed oil or no shortening gives fairly impermeable bread. Permeability of bread by air is due to the rupture of the cells during the latter stages of expansion of the loaf after starch swelling has made the dough less fluid by taking up water. When the fluid properties of the dough mass are so decreased that the cells can no longer extend themselves and remain intact, the cell wall must rupture to relieve the pressure. The permeability to air of the resulting baked bread is thus increased. Apparently where no shortening is used or liquid fat added, the cell structure, during heating, becomes so porous at the points where these liquid fats are located that the gases escape without breaking the cell wall and the resulting bread has low permeability by air. In contrast, when regular shortenings or hard fats are used, the cells become less porous, the escape of gas is prevented, and the force breaks the cell wall. Possibly this breaking of the cell wall in the latter case is helped

by the incomplete homogeneous incorporation of the semisolid fats indicated by the gluten washing experiments. The fats may lie across the cell wall and promote the breaking of the cell.

The effect of increasing the permeability by mineral oil in Table II supports the latter hypothesis, for here the oil is a liquid which does not blend readily with any of the natural oils in the dough, thus being forced to find some other location. This doubtless would lead to points of weakness in the cell wall and disruption of the cell. This mineral oil bread, which has the smallest volume, actually has the greatest permeability of those tested.

Although we have as yet made no detailed studies, it is apparent to all observers in this laboratory that breads which show high air permeability can always be rated tender breads and those which are impervious to air are more tough in character. This indicates that increased tenderness in bread, where the composition remains the same, is due to the disruption of the cell wall by the gas pressure in the baking process. The greater this cell wall breakage, the more tender the bread.

Discussion and Summary

In 1939 we observed that though semisolid fats all gave the usual improvement in bread quality, liquid fats did not when used in the same manner. Our experiments indicated that doughs containing liquid fats or those containing no shortening, when heated, begin their oven spring at exactly the same rate as doughs containing semisolid fats. However, the oven spring and the pressure within these first two doughs ceases abruptly. No difference in the physical properties of these three doughs could be shown. Therefore, this sudden increase in porosity of dough which semisolid fats prevent seems to be related to some difference in the properties of the solid and liquid fats themselves.

Semisolid fats are not the only fats that improve bread quality. Hard fats which possess substantially no plastic properties will, when finely pulverized and mixed with the dough through the entire mixing period, produce improvement similar or superior to that of commercial shortenings. Furthermore, when a liquid fat is supersaturated with a hard fat so that the mixture becomes cloudy, marked improvement in the baking quality of the bread is obtained over the use of liquid fats containing lesser amounts of dissolved hard fats.

When high-viscosity fats, such as castor oil; which maintain their viscosity during heating, are used, baking improvement is obtained. These observations suggest that those fats which improve baking quality have the property of plugging the pores responsible for the leakage in the cell walls. That this is the case was further indicated

by experiments with cocoanut oil. Bread doughs mixed and proofed below and above the melting point of cocoanut oil gave results that corresponded in all respects to those obtained with semisolid fats on the one hand and liquid fats on the other, thus proving that the physical state of the fat is the determining characteristic. Separation of fats from doughs during gluten washing indicated that the added fats mingle with the natural fats in the flour, thus further indicating that the effects obtained by fats in bread making are associated with the admixture of the added fat to the natural fat.

It may be argued, in the case of semisolid fats, that improved effects obtained are due to the liquid flour fat being moved out of location in the dough and taken up by the semisolid fat, thus preventing the leaks. This argument appears to us to lack validity because the removal of the fat would not remove the hole from the dough film. Also this removal theory is not supported by the character of hard fats recovered by gluten washing. These fats were only slightly wetted by the natural fats. Furthermore, in gluten washing when dough is made with liquid fats a very small amount of the liquid fat is recovered. It possesses a high iodine point, indicating nearly complete blending of the liquid fat with the natural fat of the flour and retained there as shown by its presence in the gluten. It is difficult to explain the effects obtained by a supersaturated solution of solid fat and liquid fat or the effects of castor oil by the removal theory. This theory has therefore been discarded by us.

Semisolid and hard fats produce a bread texture which is highly permeable by air, indicating that the cell structure is largely disrupted. Such breads are always more tender. These results can be explained by the assumption that such fats are not completely emulsed and blended with the natural fats in the dough and remain in masses which weaken the dough film in many spots. This characteristic of semisolid fats may produce the changes in dough consistency which lead many workers to consider the effects of fats as one of lubrication. The lubrication theory, however, is untenable in view of the effects of hard fats and the lack of effect of liquid fats which are themselves good lubricants.

Literature Cited

- Baker, J. C.
1939 The permeability of bread by air. *Cereal Chem.* **16**: 730-733.
— and Mize, M. D.
1939 Effect of temperature on dough properties. II. *Cereal Chem.* **16**: 682-695.
1941 The origin of the gas cell in bread dough. *Cereal Chem.* **18**: 19-34.
Bohn, L. J., and Bailey, C. H.
1937 Effects of fermentation, certain dough ingredients, and proteases upon the physical properties of flour doughs. *Cereal Chem.* **14**: 335-348.

- Fisher, E. A., and Jones, C. R.
1932 The use of oils, fats, and emulsions of oils and fats in bread-making. National Association Review, April 1932.
- Heald, W. L.
1937 Effect of different types of shortening on bread. Cereal Chem. 14: 481-488.
- Platt, W.
1923 The act of shortening in baking together with a plea for more physical tests on baked goods. J. Am. Assoc. Cereal Chemists 8: 154-160.
- and Fleming, R. S.
1923 The action of shortening in the light of the newer theories of surface phenomena. Ind. Eng. Chem. 15: 390-394.
- Sullivan, B.
1940 The function of lipids in milling and baking. Cereal Chem. 17: 661-668.

CHEMICAL FACTORS AFFECTING THE BAKING QUALITY OF DRY MILK SOLIDS. I. CORRELATION OF pH AND BAKING SCORE¹

U. S. ASHWORTH, N. S. GOLDING, G. H. FARRAH² and D. D. MILLER²

Division of Dairy Husbandry, Agricultural Experiment Station, Pullman, Washington

(Received for publication June 23, 1941)

Within the past 12 years the use of dry milk solids in the baking industry has increased nearly 300% as shown by the American Dry Milk Institute (1939). In spite of this increase it is regrettable that not all dry milk solids are of uniform good quality and can be used for baking. To offset this difficulty baking tests have to be made on samples of each batch to prevent a poor baking product from being sold.

The reasons for the variability in the baking quality of dry milk solids is not known, although many properties of the dough mixture and of the dry milk solids have been investigated. These investigations do not cover what would appear to be a necessary preliminary study of the problem, namely, whether or not the milk before processing had undergone fermentation. Such fermentation would be expected to change the pH of the dry milk solids from the natural narrow limits of normal milk as indicated by Caulfield and Riddell (1936) and Lisk (1924). Furthermore, if fermentation should play a part in this defect it is to be expected that it would be of a seasonal nature.

This study was rendered possible by the cooperation with us of the Consolidated Dairy Products Company of Seattle who maintain a baking laboratory to test the dry milk solids they sell from their co-operating manufacturing plants. Thus the samples supplied by them

¹ Scientific Paper No. 488, College of Agriculture and Agricultural Experiment Station, State College of Washington.

² American Dry Milk Institute Research Grant, 1938 to 1940. A part of the data used here were submitted by G. H. Farrah in partial fulfillment of the work required for the degree of Master of Science, 1940.

were of known baking score and represent dry milk solids manufactured between June 9, 1939, and October 15, 1940. With a few exceptions heat treatment of 180° to 190°F for one-half hour, followed by a spray process of drying, was used in their manufacture. There were 3,170 samples in all.

The object of the work reported in this paper was to determine whether there is any correlation between the normal pH of dry milk solids and their baking score, and also to find whether the variations in pH and baking score are seasonal in nature.

Methods

The experimental baking and scoring of the loaves were done by the Consolidated Dairy Products Company at Seattle. They used a standard baking formula and the loaves were compared with a control baked at the same time and scored at 89.75. The instrument used for the pH determinations was a Beckman glass electrode pH meter laboratory model G. This instrument was found to be very satisfactory.

In making the pH determinations 10 one-gram samples of dry milk solids were weighed into test tubes and each shaken for 30 seconds with 9 ml of distilled water. Between 5 and 10 minutes were allowed for hydration of each sample. Because of the evidence of protein film formation on the glass electrode as noted by Parks and Barnes (1935), the electrode was checked against a standard potassium acid phthalate buffer after every fifth sample. The pH values were reproducible to 0.02 pH units.

The samples were weighed on a Torsion Dairy Scale with a sensitivity of 0.01 g. The effect of errors in weighing was determined and found negligible. The effect of differences in the periods of time allowed for hydration of the samples was also investigated. Samples of dry milk solids were reconstituted with water at 9°C. A 5-ml portion was immediately warmed to 24°C and its pH determined. The remainder of the sample was kept at 7°C in order to prevent bacterial action. At various time intervals the pH of the mixture was determined in a similar manner. During the first 15 minutes there was no change. After one hour the average pH had increased about 0.03 unit. After five hours it had increased 0.05 to 0.10 unit.

Experimental Results

The frequency distribution for pH is given in Figure 1. The mean pH of all samples was $6.476 \pm .00080$. The distribution about the mean is approximately normal as shown by the figure. The distribution of baking scores, also shown in Figure 1, is not normal because

most of the samples fell close to the upper limit of the baking score range. The mean baking score was $89.17 \pm .0078$. About 80% of all the samples had baking scores between 89.00 and 89.75. A comparison of the variability of the two measurements can be had from their coefficients of variation, which for pH is 1.03% and for baking score 0.73%.

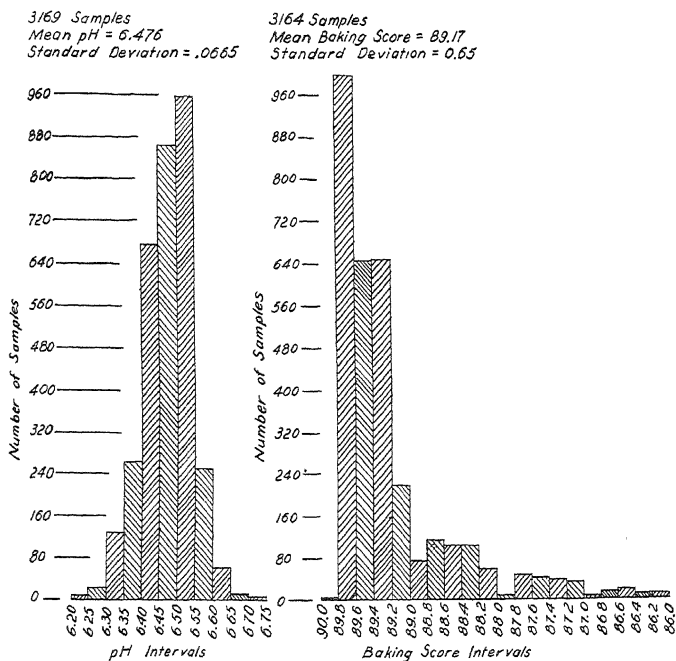


Fig. 1. Distributions of pH and baking scores.

One of the factors thought to have a possible influence on the baking score and pH of the dry milk solids is the geographical location of the dairies from which the milk originated. No very extensive study of this problem could be made, however, because all the samples placed at our disposal came from plants located in northwestern Washington except those coming from a plant in southwestern Idaho, flume No. 7. Table I shows for each flume (unit of operation in the manufacturing plant) the mean pH and baking score together with their probable errors. There seems to be a definite association of low baking scores with high pH values. The curve secured is very similar to that of Figure 2.

TABLE I
AVERAGE BAKING SCORE AND pH FOR EACH FLUME

Flume number	Approximate location	No. of samples	Average pH	Average baking score
2	N. W. Wash.	741	$6.480 \pm .0016$	$89.20 \pm .016$
3	N. W. Wash.	337	$6.484 \pm .0021$	$89.11 \pm .021$
5	N. W. Wash.	600	$6.515 \pm .0014$	$89.04 \pm .021$
6	N. W. Wash.	731	$6.445 \pm .0013$	$89.20 \pm .017$
7	S. W. Idaho	728	$6.468 \pm .0018$	$89.26 \pm .012$

Figure 2 shows for all our data baking score plotted against the pH of the dry milk solids used in the baking. The numerals beside each point indicate the number of samples averaged. The trend seems to be slightly upward between the pH limits of 6.32 and 6.47. However, when treated statistically this was found not to be significant. In other words, the pH of the dry milk solids, when between 6.32 and 6.47, had no effect on their baking score. When the pH limits were extended beyond 6.47 (practically the mean value) a very significant lowering of the average baking score was observed.

To show more clearly the significance of this point, Table II was prepared. It divides the data into six baking-score groups and shows the percentages of the various groups that have a pH above the mean.

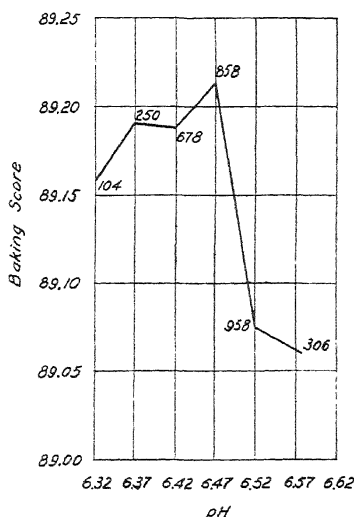


Fig. 2. Correlation of the baking score with pH. The numbers of samples averaged are indicated at each point.

TABLE II
PERCENTAGE OF SAMPLES WITH pH ABOVE 6.5

Baking score	Total number of samples	Number with pH above 6.5	Percent above pH 6.5
Above 89.5	998	449	44.8
89.0 to 89.5	1521	506	33.2
88.5 to 88.9	195	86	44.1
88.0 to 88.5	250	109	43.6
87.0 to 87.9	162	77	47.5
Below 87.0	43	37	86.1

In the group having baking scores below 87.00 nearly twice as great a proportion had pH values above 6.50 as any other group.

A distinct trend of pH with the season of the year is shown in Figure 3. The average pH values for 20-day intervals were calculated and plotted against time. During the months of July, August, and

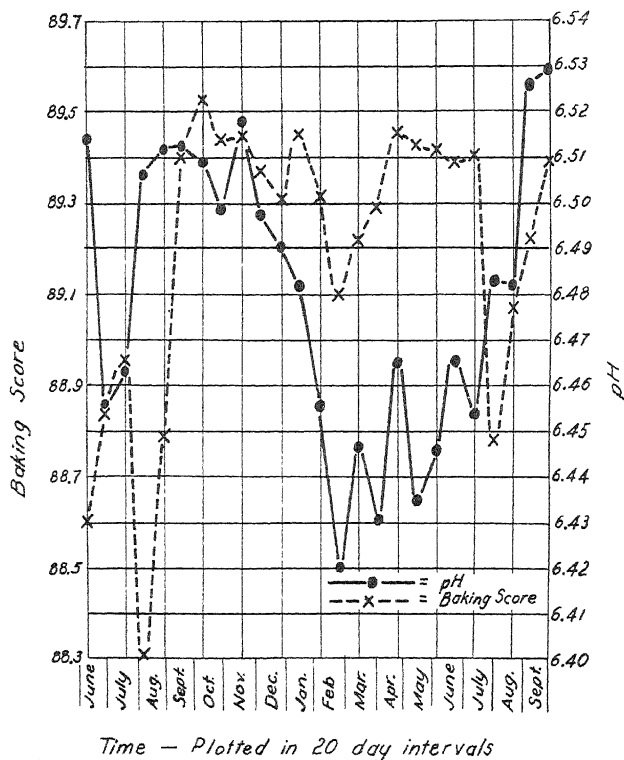


Fig. 3. Seasonal variations of pH and baking score.

TABLE III
CORRELATION COEFFICIENTS OF BAKING SCORE WITH pH

Date limits of manufacture of powder	pH limits 6.00-6.49		pH limits 6.45-6.79	
	Number of samples	r_{xy}	Number of samples	r_{xy}
<i>1939</i>				
June 9-June 28	23	+ .135	83	- .134
June 29-July 18	35	+ .250	42	- .460†
July 19-Aug. 7	56	+ .034	62	- .024
Aug. 8-Aug. 27	44	- .049	98	- .201*
Aug. 28-Sept. 16	47	- .004	138	+ .849†
Sept. 17-Oct. 6	64	+ .341†	135	- .116
Oct. 7-Oct. 26	61	- .202	115	- .196*
Oct. 27-Nov. 15	69	- .052	117	+ .050
Nov. 16-Dec. 5	47	- .122	128	- .005
Dec. 6-Dec. 25	70	+ .060	123	- .082
Dec. 26-Jan. 9	34	- .297	53	+ .128
<i>1940</i>				
Jan. 10-Jan. 29	87	+ .077	101	- .057
Jan. 30-Feb. 18	113	- .041	73	+ .318†
Feb. 19-Mar. 9	128	- .080	24	+ .118
Mar. 10-Mar. 29	120	- .073	62	+ .129
Mar. 30-Apr. 18	116	+ .080	39	- .154
Apr. 19-May 8	117	+ .074	101	- .243*
May 9-May 28	122	+ .065	68	- .152
May 29-June 17	122	+ .073	74	- .510†
June 18-July 7	120	- .002	114	+ .068
July 8-July 27	135	- .064	114	+ .126
July 28-Aug. 16	116	+ .118	139	- .136
Aug. 17-Sept. 5	28	- .099	32	+ .310
Sept. 6-Sept. 25	14	- .249	40	+ .289
Sept. 26-Oct. 15	5	+ .129	30	+ .239
June 9-Aug. 27, 1939	—	—	285	- .205†
Mar. 30-June 17, 1940	—	—	282	- .265†

* Correlation coefficient significant (5%).

† Correlation coefficient highly significant (1%).

September, 1939, there was an increase in the average pH which reached a maximum of 6.51 in October. From then on there was a sharp decline to an average of 6.43 by the first of March, followed by a gradual increase during the spring and summer. These various trends have been tested statistically and found significant.

In a similar manner the averages of the baking scores for 20-day intervals were calculated, plotted against time and the significance of the various trends determined statistically. The downward trend at the start was found to be quite significant (June, July, and August, 1939). Then there was an abrupt rise during September which took the average baking score from a low of 88.3 to a high of 89.5 in October, 1939. Such a high average shows that there were practically no poor baking samples manufactured during that period. During the winter months there was a gradual, but significant, downward trend to an average of 89.1 in February, 1940. The average baking score then

took a sharp upward turn, leveling off at an average score of a little above 89.4 during April, May, June, and July. For the period July 28 to August 16, 1940, the average baking score had dropped to 88.8. This was followed by a rise during September. Thus in both years poor baking quality was associated with the hottest part of the year at which time the pH was above the average and rising.

A comparison of the curves in Figure 3 suggests that there is no simple relationship between pH and baking score. Figure 2, however, shows a definite association between pH values and low baking scores. In order to get a more accurate picture of the relationship between pH and baking score the effect of season was eliminated. This was done by determining two correlation coefficients for each 20-day interval, one for the pH limits of 6.00 to 6.49 and the other for the pH limits of 6.45 to 6.79. The results are shown in Table III.

Most of the correlation coefficients in Table III are not significant. Those that are slightly significant are designated by a single asterisk; those that are highly significant are designated by a dagger. The algebraic mean of the correlation coefficients for all 25 groups is $+.01201$ for pH limits 6.00 to 6.49 and $+.0051$ for pH limits 6.45 to 6.79. Neither value is significant statistically.

Discussion

The pH of fresh milk from normal udders falls within the range of 6.22 to 6.77 as reported by Sommer and Matsen (1935). Our results obtained from the composites of many cows would be expected to show a much more narrow limit. The mean value of our 3,169 samples was found to be 6.476 (Table I). The calculated mean of the 386 samples reported by Sommer and Matsen was found to be 6.476. The identity of these means indicates that the tendency toward acid or alkaline fermentation, if it occurred in the milk used for dry milk solids, was very nicely balanced.

It is possible, but unlikely, that the pH values within the range studied will themselves affect the baking quality. St. John and Bailey (1929) found the buffering action of dry milk solids on bread doughs to be appreciable but they observed an accelerated fermentation rate due to the milk and concluded that there must be a factor in the milk which compensated for the decrease in acidity.

The direct correlation of pH with baking score for all the data shows that the more alkaline samples of milk are generally associated with the lower baking scores. Nevertheless when the effect of season was eliminated by determining the correlation coefficient for each 20-day period and averaging the resulting values, all significance was lost in the correlation between baking score and pH (Table III).

The effect of the season of the year on the pH of milk has not been

studied very extensively. According to Caulfield and Riddell (1936), the most important factor influencing the pH of normal milk is the stage of lactation. With the advance of lactation the pH increases to the alkaline side. It was not possible for us to study directly the effect of the stage of lactation on the baking quality of the milk because our samples represented composites of many animals.

The feed of the cow is a possible factor influencing the pH of the milk. The work of Sommer and Hart (1921) and others indicates that such an effect is unlikely, although the problem is not settled in the minds of many workers in the field.

In our work it is interesting to note that the minimum value for the pH (averaged in 20-day intervals) occurred in February and the maximum value occurred in August. If lactic-acid-forming bacteria were entirely responsible for the lower values, such values should occur in the warmer season.

Summary

An examination of 3,170 samples of dry milk solids for pH and baking score was made.

There was no significant effect of the pH of dry milk solids on the baking quality of the doughs made from them.

Dry milk solids with poor baking quality were produced in the main during the latter part of July and through August for both years studied. The pH at this time of the year was above average.

Acknowledgments

Thanks are tendered to the Consolidated Dairy Products Company, Seattle, and their staff for their unflinching cooperation in supplying the samples and baking scores of the same; to the American Dry Milk Institute, Chicago, for providing a large part of the funds for the work; also, to the Research Department of the Federal Land Bank, Spokane, who through the assistance of Dr. H. Ashley Weeks enabled the punch card system to be used for sorting the data.

Literature Cited

- American Dry Milk Institute, Inc.
1939 101 questions and answers about dry milk solids (4th ed.). Chicago, Ill.
Caulfield, W. J., and Riddell, W. H.
1936 Some factors influencing the acidity of freshly drawn cow's milk. *J. Dairy Sci.* 19: 235.
Lisk, H.
1924 A quantitative determination of the ammonia, amino nitrogen, lactose, total acid, and volatile acid content of cow's milk. *J. Dairy Sci.* 7: 74-82.
Parks, L. R., and Barnes, C. R.
1935 Application of the glass electrode to dairy products. *Ind. Eng. Chem. (Anal. Ed.)* 7: 71-72.
Sommer, H. H., and Hart, E. B.
1921 Grading milk by the acid test: influence of acids in the ration on the acidity of milk. *J. Dairy Sci.* 4: 7-11.
Sommer, H. H., and Matsen, Helene
1935 The acidity of milk and dairy products. *Wisc. Agr. Exp. Sta. Res. Bul.* 127.
St. John, J. L., and Bailey, C. H.
1929 Effect of dry skimmilk on the fermentation and hydrogen-ion concentration of doughs. *Cereal Chem.* 6: 51-59.

FACTORS WHICH INFLUENCE THE PHYSICAL PROPERTIES OF DOUGH. IV. THE EFFECTS OF SURFACE ACTIVE AGENTS ON THE CHARACTERISTICS OF THE CURVES MADE BY THE RECORDING DOUGH MIXER¹

C. O. SWANSON and A. C. ANDREWS

Agricultural Experiment Station, Manhattan, Kansas

(Read at the Annual Meeting, May 1941)

Wide variations in dough development curves made by the recording dough mixer were reported by Swanson (1939). What factors cause these variations? Why is one flour wetted more rapidly by water than another? The steeper rise of the curve is apparently due to the more rapid wetting. Why is there a more apparent breakdown, shown by the steep downslope and narrowing of the curves of Chiefkan and Early Blackhull, than with the more rounded top and more gradual downslope of Turkey, Tenmarq, and the spring wheat flours? Why are some flours more sensitive to amount of mixing than others? That the amount of protein and absorption influence the pattern of the curves has been shown by Swanson (1941) and also by Larmour, Working, and Ofelt (1939).

It was thought that the use of surface-active agents, because of their known effects on the interfacial tension of water films, which might also manifest themselves in dough, would throw light on some of these questions. Before proceeding to the experimental evidence, some theoretical consideration on which the investigations were based will be given.

Gluten Structure in Dough

In a well mixed dough, the gluten seems to exist as a continuous three-dimensioned network (Swanson, 1925, 1937). It is probable that on mixing in the pull-fold-repull type of mixer such as the National-Swanson-Working recording mixer, the protein molecules assume the fibrillar type. In the dough which has been mixed just enough for the water to envelop all the flour particles, the protein particles which form the wet gluten are arranged in a heterogeneous pattern. With the pulling, folding, and repulling, the fibrillar protein molecules become oriented into a more or less parallel pattern, arranged as a three-dimensioned gluten network in which the starch and other substances are enmeshed. The increasing resistance to the moving pins, shown in the upward swing of the pen, is due to the gradual perfecting of the parallel pattern. The

¹ Contribution No. 76, Department of Milling Industry, and No. 265, Department of Chemistry.

perfecting of this parallel pattern is shown in the increasing smoothness of the dough, the appearance of which may be taken as an indication of the probable optimum amount of mixing.

Water Films in Dough

In a well mixed dough, the water films which cover the starch granules and the strands of the three-dimensioned gluten mesh form a continuous phase. The water molecules next to the actual surfaces of the starch and gluten are so strongly adsorbed that they have no freedom. Layers of water molecules superimposed on these have more and more freedom as the distance from the surface of the solids increases until in the outer layers of the water films the freedom approaches that which exists in a body of water. It is this freedom of the water molecules in the outer portions of these films which allows the sliding of the gluten particles on each other and on the starch. The formation of a protein film over the starch granules has been suggested by Markley (1938). The thickness of the water films varies with the amount of absorption.² The more water used in absorption, the greater the freedom of the water in the outer layers and hence the less resistance to the pins which pull, fold, and repull the dough. For this reason the height of the curve will vary inversely with the amount of absorption water (Swanson, 1941). That both the quantity and quality of protein, as in Tenmarq and Chiefkan, influence the pattern of the curve was shown by Larmour, Working, and Ofelt (1939). The quantity of protein directly influences the density of the gluten mesh since with a flour of higher protein there is more material to form strands.

Effect of Interfacial Tension

The viscosity or internal friction of the water molecules in these liquid films will have an influence on the ease of sliding of the dough particles on each other and hence on the curve characteristics. The addition of substances that lessen the adhesional tension of the water for the gluten would influence the ease with which these gluten fibrils move over one another. Less strongly adsorbed water would allow greater freedom of movement, which would be manifest in a longer time of dough development and also a prolongation of the period of breakdown. Consequently the presence of substances that lower the interfacial tension between water, gluten, and starch should have a marked effect on the mixing behavior of the dough as shown in the curve characteristics.

² The term *absorption* will be used for reference to the amount of water used to mix a dough of desired consistency or as understood in the baking trade.

Surface-Active Agents

Many substances have been developed recently that have the property of greatly reducing the surface tension of water and becoming largely adsorbed at interfaces. These are commonly known as "wetting agents" and also as surface-active or interfacial-tension-reducing agents. Some agents such as alcohol and organic acids that would be included in this classification are unsuitable for use with flour because they have a disintegrating effect on the gluten molecules.

A survey of commercial products manufactured for various purposes, particularly those of hydrophilic nature, revealed a large number suitable for this investigation. It was first thought that the main effect of these surface-active agents would be on the interfacial tension of the water films in the dough. It was soon found that their action was not limited to the reduction of the interfacial tension of such water films. The curves obtained made it appear that the surface-active agents also have an effect on the structure or arrangement of the protein substance. This, however, may or may not be related to the surface-tension reduction of the water.

Nature of Surface-Active Agents

"Surface-active agents are polar compounds and their activity is dependent on the ability of their molecules to become oriented and adsorbed at an interface," according to a definition by Bartell (1939). Ordinarily, molecules of surface-active agents are composed of lyophobic and lyophilic groups. One part of the molecule may consist of a straight or branched hydrocarbon chain or chains which are more or less lyophobic. To this part is usually attached solubilizing groups such as $\text{—SO}_3\text{H}$, $\text{—SO}_3\text{Na}$, $\text{—PO}_3\text{H}_2$, —COOH , —OH or their corresponding salts which are more or less lyophilic. Since water was the solvent used in this study, *hydrophilic* is the specific term. Because of this dual nature of the molecules they may become oriented at an interface.

The molecules of surface-active agents will have a tendency to locate themselves on the surface of the water in such positions that their hydrophilic groups are directed into the water while their hydrophobic groups extend above the surface. In this position they serve to tie both phases together and thus tend to reduce the abruptness of the phase transition. Whether the reduction of surface tension of this free water in the dough would be the same as when the surface-active agents are in a water solution can of course not be predicted.

General Procedure

Some of these agents were on hand and others were obtained from the manufacturers. Acknowledgment is hereby made for their generous

donations. Only those agents which were readily soluble in water were suited for this work and solutions from 1% to 10% were found to be convenient. From the many agents known to exist, about 35 were selected, and two flours, Tenmarq and Chiefkan, each having medium protein percentages, were used in the preliminary trials. The general procedure was to make trial curves with 100 mg of the agent dissolved in the amount of water used for mixing the dough from 35 g of flour. From the appearance of this trial curve it was possible to decide on the varying amounts of the material that should be used for groups of three or four curves. The smallest amounts used were such as to show a distinct effect on curve characteristics when compared with the curve made from flour and water. The largest amounts were such as to show very marked effects and still retain the main pattern obtained from sound flour.

The Three Main Measures of Curve Characteristics

The three most significant measures of the main characteristics of a curve are: minutes for the recording pen to reach the top of the curve; height of this top, measured in units on the chart; and angle between the rise and fall of the curve. Reference to Figure 1, which shows one curve from Turkey and one from Chiefkan together with the skeleton outline of these curves, will help in following this description. The distance between the curved lines on the chart paper and the rate of move-

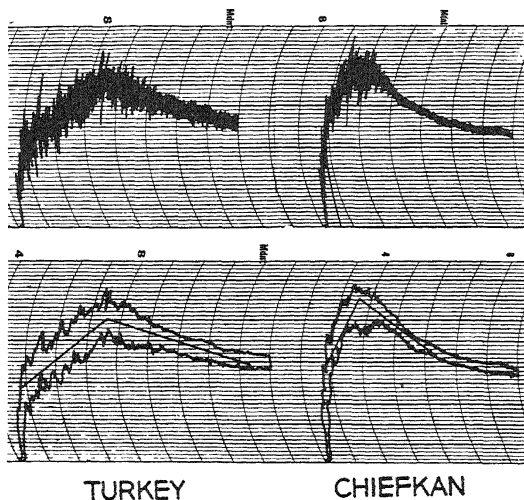


Fig 1. Curves of Turkey and Chiefkan and their skeletons, illustrating method of describing curve characteristics

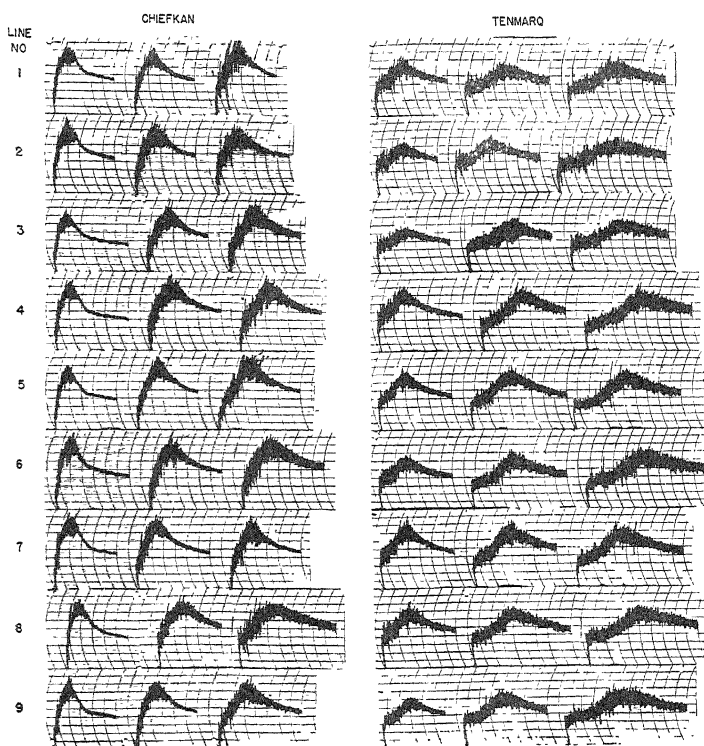


Fig. 2. Effects of surface-active agents.

Agent	Line No.	Chiefkan—agent added			Tenmarq—agent added		
		mg	mg	mg	mg	mg	mg
Aerosol AY	1	0	40	70	0	40	70
Aerosol OT	2	0	70	100	0	20	60
Dupenol PC	3	0	70	100	0	70	100
Gardinal WA	4	0	100	170	0	100	200
Gardinal WA Flakes	5	0	100	200	0	100	200
Santomerse S	6	0	100	200	0	100	200
Penetrant W 846	7	0	70	100	0	70	100
Tergitol Pen. 4	8	0	100	200	0	60	150
Tergitol Pen. 7	9	0	70	140	0	70	140

ment are such that the time required for the pen to go from one curved line to the other is so near one minute that for practical purposes this is the time unit. The bold horizontal lines divide the chart into ten units and the light lines subdivide these units into five each. Hence, the height of the curve can be measured in units from 1 to 10 and in decimals of these units. The angles made by the up-and-down slopes are

measured by drawing a line in the center of the upslope of the curve and also in the center of the first part of the downslope. The angle which these two lines make near the center of the top of the curve is measured by a protractor and the angle is stated in degrees. This is essentially the method used by Shellenberger (1940).

There are other characteristics in the curves which are significant, such as the general width, the outline of the top, such as rounded or sharp, the curvature of the downslope, and the rate of narrowing of the last portion of the curve (Swanson, 1937). These are used in the visual judging of the curves, but in this discussion the three points mentioned,

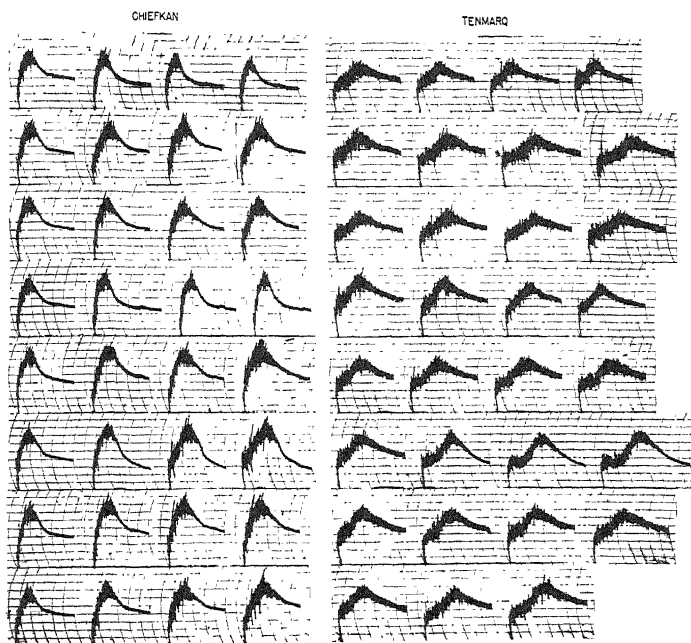


Fig 3. Effects of surface-active agents

Agent	Line No.	Chieffkan—agent added				Tenmarq—agent added			
		mg	mg	mg	mg	mg	mg	mg	mg
Aerosol DGA	1	0	70	100	140	0	70	100	140
Aerosol NAD	2	0	70	100	140	0	70	100	140
Dupenol OS	3	0	100	200	300	0	100	200	300
Loupenne	4	0	100	140	200	0	100	140	200
Tetra K Phos.	5	0	40	70	100	0	40	70	100
Triton S-18	6	0	100	20	300	0	100	200	300
Wetsit single	7	0	40	70	100	0	40	70	100
X Dry size	8	0	100	200	300	0	100	200	

time of development, height, and angle made by the slopes, will be used in describing modifications caused by the presence of the surface-active agents in the dough.

Effects of the Surface-Active Agents

A study of the curves obtained with the use of about 35 surface-active agents showed that they could be divided into two general classes: one in which the effects were very pronounced and the other in which the effects were not so pronounced. Typical curves in which the effects of the agents were very pronounced are shown in Figure 2 and the legend gives the names and the amounts used for each curve. The small variations in the control curves made with flour and water shown in the first column for each variety are due to the fact that these curves were made on different days with only such atmospheric control as is found in the average laboratory.

TABLE I
SURFACE TENSIONS OF SOLUTIONS OF SOME SURFACE-ACTIVE AGENTS

For curves in Figure 2			For curves in Figure 3		
Agent	Amt. per 21 ml H ₂ O	Surface tension	Agent	Amt. per 21 ml H ₂ O	Surface tension
	mg	dynes/cm		mg	dynes/cm
Aerosol AY	70	28.3	Aerosol DGA	140	27.5
Aerosol OT	60	29.9	Aerosol NAD	100	33.1
Dupenol PC	100	37.7	Dupenol OS	300	31.1
Cardinal WA	170	30.6	Loupeno	100	30.7
Cardinal EA Flakes	200	30.6	Tetra K Phos.	100	67.1
Santomerse S	150	36.1	Triton S 18	300	31.0
Penetrant W 846	100	34.8	Wetsit single	100	33.4
Tergitol Pen. 4	100	37.6	X Dry Size	300	36.9
Tergitol Pen. 7	140	28.7			

The curves in Figure 2 show that the general effects of the surface-active agents are longer time for development with consequent less-steep upslope, general wider width of the curves, less steep downslope, and a decreased tendency of the curves to become narrow in the last portions. This last effect is particularly true of the curves from Chiefkan, but not so pronounced on Tenmarq. On Chiefkan the influence is so great that the curve characteristics associated with this variety are obliterated. Thus the third column of curves under Chiefkan have a pattern very much like that obtained from Turkey or Tenmarq.

The curves in Figure 3 show that the effects of the surface-active agents used for these curves were much less than those used for the

curves in Figure 2. The patterns of the curves for both Chiefkan and Tenmarq were not greatly altered, although the amounts used for the curves in the fourth column were in several cases larger than those used for the curves in Figure 2.

Surface Tensions of Solutions of Surface-Active Agents

Surface-tension measurements were made with the du Noüy ring method in order to determine whether the differences in the curves of Figure 2 and Figure 3 were due to variations in surface tension. Representative figures given in Table I show that the surface tension of the solutions of these agents were, in general at least, half that of ordinary distilled water, which was 71.9. The agents used for the curves in Figure 2 had about the same effect on surface tension as those used for Figure 3. These figures show that reductions in surface tension were not of first importance. If they had been, the curves in Figure 2 would have been more similar to those in Figure 3.

TABLE II

EFFECT OF INCREASING CONCENTRATION OF AEROSOL OT ON SURFACE TENSION

Milligrams per 21 ml	Surface tensions
	<i>dynes/cm</i>
0	71.9
10	35.8
20	30.8
30	29.9
40	29.5
50	29.5
60	29.5

Furthermore, increasing the amounts of the surface-active agents had a cumulative effect as shown by the curves in Figure 2. As is well known, surface-active agents will decrease the surface tension of water a definite amount, but when this reduction has reached a certain point there will be no further effect. As shown by the figures in Table II, the presence of 10 mg of Aerosol OT in 21 ml of water reduced the surface tension about one-half. Doubling this amount made a further reduction only about one-seventh as great as the first 10 mg. Adding 30 mg or more produced little or no further reduction in surface tensions. However, as shown in Figure 2, and as will be shown later, each additional amount of surface-active agent produced marked effects on the curves.

Effect of the Surface-Active Agent Aerosol OT on Curves of Flours of Varying Protein Content

In the further trials with the effects of surface-active agents, the work was limited to the use of Aerosol OT. While this did not have quite as great an effect as some of the other agents, nevertheless, as seen from the second row of curves in Figure 2, the modifications due to this agent were notable. A particular advantage in the use of this agent is that it is patented and hence its formula is known.

The flours used in these trials were available samples of varying protein content from each of the varieties: Tenmarq, Cheyenne, Chiefkan, and Clarkan. The protein percentages of these samples are given in Table III. The flours were milled from blends composed of a number

TABLE III
PROTEIN IN FLOURS FROM FOUR VARIETIES

Variety	Protein content					
	cc	%	%	%	%	%
Tenmarq	15.3	13.3	11.8	10.8	9.3	7.9
Cheyenne	—	13.2	11.2	10.4	9.2	—
Chiefkan	14.6	13.1	—	10.8	9.3	8.7
Clarkan	—	12.8	11.3	9.9	8.7	8.3

of wheat samples of varying protein contents. The wheats were grown in different parts of Kansas.

One problem with flours of varying protein content is to obtain a satisfactory range in water absorption for the curves. It was desired that the upper parts of the curves from the highest-protein samples should be near the top of the chart. The amount of water needed for this height was determined by trials. It was found that as the protein content became lower the most desirable curves from flour and water were obtained by decreasing the absorption 1.5% for each 1% decrease in protein. This held for the higher-protein samples, but as the protein content decreased the effect on absorption became less. The same absorptions as were used for the flour-and-water curves were also used when the surface-active agent was present in the water.

The curves obtained on the flours of varying protein content from the four varieties are given in Figure 4. The first row of curves for each variety was made with flour and water and the second with flour, water, and 50 mg of the surface-active agent, Aerosol OT. The measurements, minutes to reach the top, height in units on the chart, and angles of the up-and-down slopes are given in Table IV.

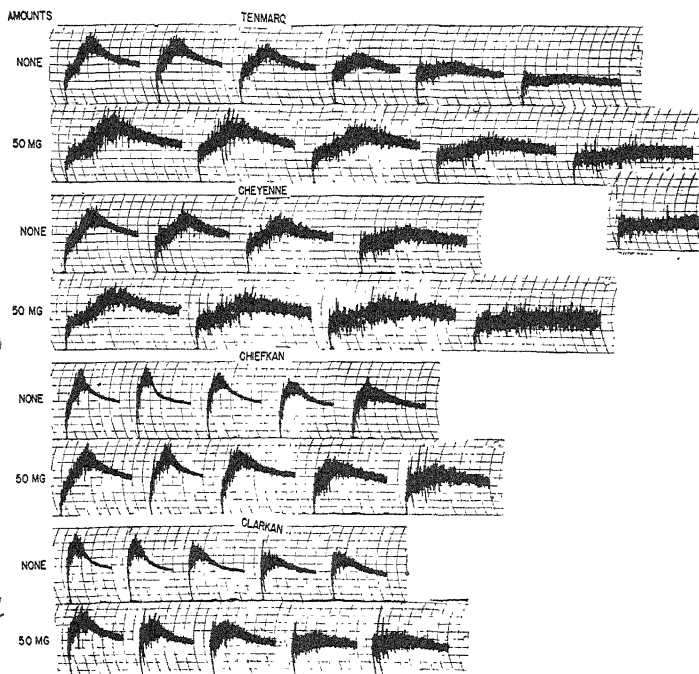


Fig. 4. Effect of surface-active agent on curves of flours of decreasing protein percentages.

First line of curves under each variety: flour + water.

Second line of curves under each variety: flour + water + 50 mg Aerosol OT.

	TENMARQ					
Protein, %	15.3	13.3	11.8	10.8	9.3	7.9
Absorption, %	65.0	62.0	59.8	58.3	56.8	56.0
	CHEYENNE					
Protein, %	13.2	11.2	10.4	9.2		
Absorption, %	61.0	58.0	57.0	57.0		
	CHIEFKAN					
Protein, %	14.6	13.1	10.8	9.3	8.7	
Absorption, %	65.0	63.0	59.3	57.8	57.5	
	CLARKAN					
Protein, %	12.8	11.3	9.9	8.7	8.3	
Absorption, %	58.0	55.8	54.3	53.8	53.5	

The heights of the flour-and-water curves shown in Figure 4 become progressively less as the protein content of the flour decreases. The time required to reach the top was shortest for Clarkan, somewhat longer for Chiefkan, longest for Cheyenne, with Tenmarq somewhat shorter than for Cheyenne. The angles between the slopes increased with decreasing protein content. The time to reach the top became longer as the protein decreased.

TABLE IV
EFFECT OF SURFACE-ACTIVE AGENT ON CHARACTERISTICS OF FLOUR OF
DECREASING PROTEIN CONTENT

Curves in Figure 4.

Protein	Absorption	Rate of development		Height to top		Angles of up-and-down slope	
		Flour & water	Aerosol present	Flour & water	Aerosol present	Flour & water	Aerosol present
%	%	min	min	units	units	deg	deg
TENMARQ							
15.3	65.0	3.0	5.2	8.4	8.0	104	135
13.3	62.0	2.6	4.1	7.8	7.4	113	140
11.8	59.8	3.2	5.7	7.4	6.0	123	150
10.8	58.3	3.0	6.0	6.2	5.6	136	163
9.3	56.8	4.2	8.2	5.4	5.0	158	171
7.9	56.0	4.7	8.0	3.8	4.0	173	175
CHEYENNE							
13.2	61.0	3.5	5.6	7.8	7.6	115	135
11.2	58.0	3.6	6.1	7.4	6.6	130	155
10.4	57.0	3.9	7.4	7.2	6.2	139	165
9.2	55.0	5.1	8.1	5.8	5.2	162	172
CHIEFKAN							
14.6	65.0	2.0	3.3	8.4	8.2	87	109
13.1	63.0	1.5	2.1	8.6	8.6	74	94
10.8	59.3	1.6	2.5	8.2	8.0	102	111
9.3	57.8	1.9	3.0	7.2	6.6	105	138
8.7	57.5	2.2	4.3	6.6	5.8	135	155
CLARKAN							
12.8	58.0	1.2	2.0	8.4	8.0	82	114
11.3	55.8	1.1	2.3	7.6	6.8	102	128
9.9	54.3	1.4	2.2	7.0	6.8	113	129
8.7	53.8	1.3	3.5	5.8	5.4	145	159
8.3	53.5	1.9	3.9	6.0	5.2	145	164

The effect of the presence of the surface-active agent is shown in the second row of curves for each variety in Figure 4. The heights of the curves are slightly decreased, the time required to reach the top of the curves and the angles made by the slopes very much increased, and the general width of the curves also notably increased. The presence of the surface-active agent made the Chiefkan curves approach the characteristics of the flour-and-water curves from Tenmarq.

Effects of Increasing Amounts of Surface-Active Agent on Samples of Decreasing Protein Content

The samples shown in Figure 4 were used to test the effects of using 0, 20, 40, and 60 mg of the surface-active agent Aerosol OT, respectively, for each curve. The curves obtained from Tenmarq and Chey-

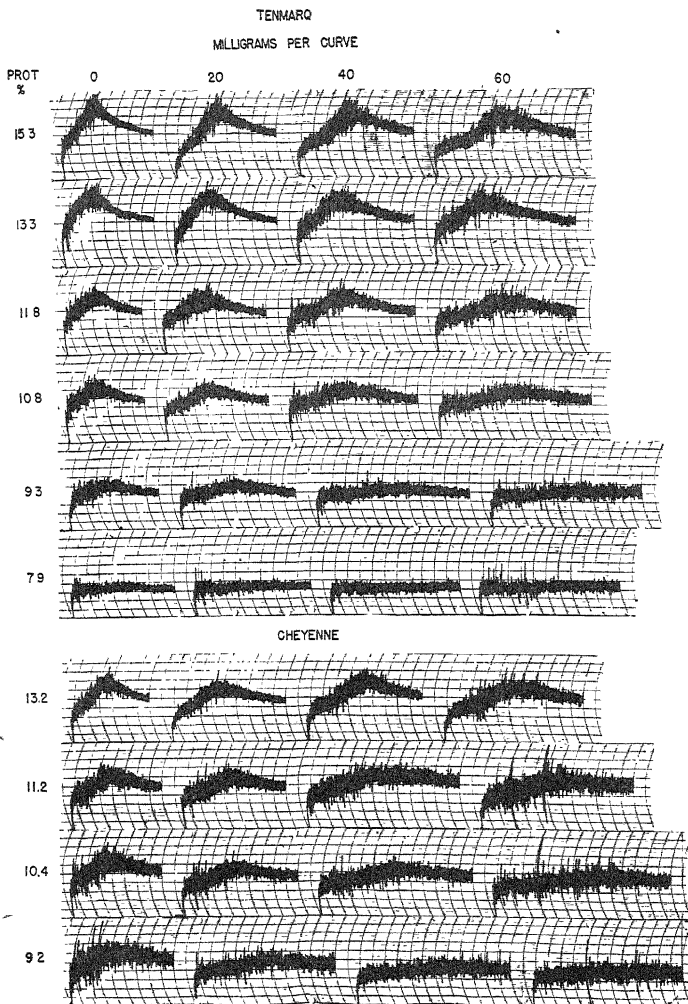


Fig. 5. Effects of increasing amounts of surface-active agent on samples of decreasing protein content—Tenmarq and Cheyenne.

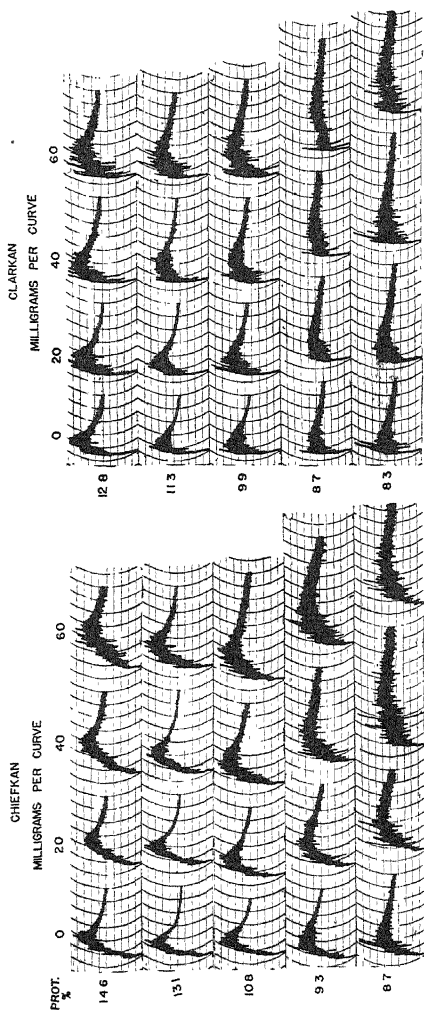


Fig. 6. Effects of increasing amounts of surface-active agent on samples of decreasing protein content—Chieffkan and Clarkan.

TABLE V

EFFECTS OF INCREASING AMOUNTS OF SURFACE-ACTIVE AGENT
ON SAMPLES OF DECREASING PROTEIN CONTENT

Curves for Tenmarq and Cheyenne are shown in Figure 5, and curves for Chiefkan and Clarkan in Figure 6.

Protein	Amt. of agent	Rate of development	Height	Angle
%	mg	min	units	deg
TENMARQ				
15.3	0	3.0	9.0	105
	20	4.0	8.2	114
	40	4.5	8.0	127
	60	6.0	7.4	141
13.3	0	2.6	8.8	108
	20	3.3	8.2	119
	40	3.9	8.0	132
	60	4.3	7.8	138
11.8	0	3.0	6.8	125
	20	4.0	6.8	138
	40	5.1	7.0	145
	60	6.2	6.4	157
10.8	0	3.0	6.6	135
	20	4.1	6.4	146
	40	5.2	6.0	158
	60	7.0	5.8	162
9.3	0	3.9	5.4	158
	20	5.5	5.4	163
	40	7.2	5.0	172
	60	8.0	4.8	176
CHEYENNE				
13.2	0	3.5	7.6	118
	20	4.5	6.8	140
	40	5.2	7.4	140
	60	6.1	6.8	153
11.2	0	3.9	6.8	142
	20	4.7	6.4	153
	40	6.5	6.8	163
	60	7.0	6.4	165
10.4	0	3.4	7.2	138
	20	5.0	6.2	155
	40	6.8	5.8	166
	60	9.0	5.6	169
9.2	0	4.6	6.6	158
	20	7.0	5.4	167
	40	7.7	4.8	172
	60	10.4	4.7	174

TABLE V—*Continued*

Protein	Amt. of agent	Rate of development	Height	Angle
%	mg	min	units	deg
CHIEFKAN				
14.6	0	2.0	8.4	87
	20	2.5	7.8	107
	40	3.0	8.0	107
	60	3.5	7.8	120
13.1	0	1.3	8.4	75
	20	1.7	8.4	82
	40	1.8	8.4	95
	60	2.2	8.8	89
10.8	0	1.6	8.4	86
	20	1.9	8.4	107
	40	2.0	8.6	97
	60	2.7	8.2	120
9.3	0	1.5	7.8	115
	20	2.3	7.8	117
	40	3.0	7.4	129
	60	3.4	7.6	136
8.7	0	1.7	6.8	126
	20	2.7	6.8	131
	40	4.0	6.4	157
	60	4.0	6.2	152
CLARKAN				
12.8	0	1.3	9.0	89
	20	1.6	8.2	102
	40	1.7	8.6	105
	60	2.3	8.4	118
11.3	0	1.2	7.4	97
	20	1.5	7.4	105
	40	1.9	7.0	121
	60	2.4	7.0	132
9.9	0	1.1	7.2	110
	20	1.6	7.2	109
	40	2.3	6.8	136
	60	2.4	7.0	134
8.7	0	1.9	5.8	149
	20	2.6	5.8	156
	40	3.0	5.6	160
	60	3.9	5.4	160
8.3	0	2.0	6.0	151
	20	2.3	6.0	151
	40	2.2	6.0	161
	60	3.2	6.0	160

enne are given in Figure 5 and the curves from Chiefkan and Clarkan are given in Figure 6. The measurements of these curves are given in Table V.

The curves for each variety are arranged in the plates so that the flour-and-water curves are first in each column, and the others follow in order of increasing amount of surface-active agent. The curves for the various rows of each variety are arranged in order of decreasing protein content. Thus the curves in the first row are from the highest-protein flour and the last row of curves from the lowest-protein flour.

Increasing the amounts of the surface-active agent lengthens the time to reach the top, enlarges notably the angles of the up-and-down slopes, and decreases the heights of the curves of Tenmarq and Cheyenne more than of Chiefkan and Clarkan.

Discussion

The surface-tension values obtained (Tables I and II) were in most instances less than half that of water. The viscosity was determined on the same solutions that were used for the surface-tension measurements given in Table I, using an Ostwald viscosimeter. All these solutions had a viscosity very little different from water alone. Hence, as accords with general knowledge, the surface tension and the viscosity of these solutions are independent of each other. A change in viscosity should be evident from the height of the curves obtained when the surface-active agents were present. Actually the heights were changed very little in comparison with the rate of development. Accordingly there must be at least another type of effect operative in the behavior of dough due to the presence of the added substance. Such might be found in the effect of increased wetting of the gluten and starch particles. Lowering of the interfacial tension and increased spreading ability would result from an increase in the adhesional tendency of water toward the gluten particles covered with the adsorbed wetting agent. In this way each gluten particle would become more completely covered with water and perhaps the molecular interstices would be penetrated to a greater extent. This might be a consequence of the interfacial tension reduction. Additional experiments are being conducted to clarify this problem.

The curves presented show that the mixing time was increased by the presence of the surface-active agent. This could be due to its specific action on the protein molecules themselves in effecting varying degrees of intramolecular changes. Such changes could be related to some form of denaturation.

Protein structure and denaturation: According to writers on proteins (Wrinch, 1937, Lloyd and Shore, 1938; Schmidt, 1938) such substances may assume various physical forms, such as globular or polyhedral, laminar and fibrillar. The models of protein molecules, according to Wrinch (1937), allow for the existence of the fibrillar or one-dimensional, the laminary or two-dimensional, and the globular or three-dimensional form. The three-dimensioned molecules are built up from laminary or two-dimensional cyclised polypeptides which in turn have been formed from fibrillar or one-dimensional molecules. The flat cyclised polypeptides may be held together in piles of two or more deep alternately by direct backbone and side chain linkages. The three-dimensional protein molecules are built by bending round the cyclol fabric so as to make a continuous space-inclosing network in the form of tetrahedrons. The fibrillar forms can pack together to make larger fibrils. In protein films the laminary form is suggested. The globular form exists usually in colloidal solution. Its occurrence in dry flour is possible and its existence in dough is assumed.

Probably a principal effect of the surface-active agent is to further promote the penetration of water into these polyhedral forms and so facilitate their change to other forms. Such changes are implied in denaturation which seems to involve structural alterations of the protein molecules. This can be done according to Lloyd and Shore (1938) by various agents including mechanical agitation, and the action of surface-active agents. Denaturation appears to be accompanied by change in molecular form such as from the globular to the fibrous. This may account for the changes in viscosity which accompany denaturation.

If some sort of globular forms are assumed, such as those suggested by Wrinch (1937), the wetting agent then acts upon this cagelike structure and ruptures it at its weakest links. Such rupture could take place at the hydrogen bonds, the -S-S linkages, and possibly even at the peptide linkages. This rupture would cause a straightening out of the structure into long fibrous patterns. These become more and more fibrous as a result of action of the surface-active agent supplemented by the continued mechanical agitation such as takes place in the bowl of the recording dough mixer. In addition to this, the protein molecule itself contains numerous chemically active groups. These may decompose, rearrange, or react with themselves or with the agent. The latter effect could be a rather prolonged one, due to the time required to disrupt the cagelike protein structures. This process in turn would produce new active groups that were previously either chemically bound into the structure or because of spatial effects were unavailable until exposed by denaturation process. The time element involved in these changes may

account for the lengthening of the period of development shown in the curves when a surface-active agent is present.

Adhesive function of water films in dough: Dough is a coherent mass because of the forces residing in the interfacial water films. These films cover the starch granules as well as the protein particles. The gluten fibrils consist of interwoven protein particles or micelles which entrap the water. Mechanical action such as results from the action of the recording mixer will rearrange the micellar structure. The net result is an increase of surface which would decrease the amount of free or increase the amount of bound water.

Effect of decrease in surface tension due to the presence of the surface-active agents: The presence of the surface-active agent in the water films of the dough promotes the increase of protein surface through their denaturing action, resulting in uncoiling the protein micelles. This spreading of the water films over greater increased protein surfaces would decrease the amount of free water and alter the viscosity of the dough. Such alterations in viscosity, which should result in greater curve heights, were not uniformly found when the surface-active agent was present. This may be due to the weakening of the protein or gluten strands as a result of denaturation by the surface-active agent.

Summary

Surface-active or interfacial-tension-reducing agents modify the characteristics of the curves made by the recording dough mixer to such an extent that the patterns associated with such varieties as Chiefkan may be made similar to the patterns of Turkey or Tenmarq. The most notable effects are to increase the time of development, increase the angles of the up-and-down slopes, and decrease the rate of breakdown of the dough. The smooth narrow characteristic of the last part of Chiefkan curves was completely obliterated.

The surface-active agents reduced the surface tension of water about one-half or more. The effects on the doughs as shown in the curves cannot be explained solely by decreases in the surface tension. The effects of the agents were greater as the concentration of the agent increased. The surface-tension reduction of water proceeds to a certain point after which further additions of the agent produce no further decrease. Some agents had very little effect on the curve patterns, yet they reduced the surface tension of water to about the same extent as those which had marked effects.

The effects of these agents on the curve patterns may be due to their ability to alter the configuration or form of the protein molecule. This change in the protein molecule may be a consequence of interfacial ten-

sion reduction by allowing greater intramolecular penetration of the water. This, together with the mechanical pull-fold-repull action on the dough, may break various bonds, changing the polyhedral molecular form through laminar to fibrillar. Those agents which do not have a marked effect on the curve patterns may have a composition which is less effective in causing this denaturation.

The curve patterns from high-protein flours were changed more than the patterns from low-protein flours. This indicates that the agents affect the protein principally and would support the view that the effects are due to some form of protein denaturation.

Literature Cited

- Bartell, F. E.
1939 Surface-active agents (a symposium). Introduction. *Ind. Eng. Chem.* 31: 31.
- Larmour, R. K., Working, E. B., and Ofelt, C. W.
1939 Quality tests on hard red winter wheats. *Cereal Chem.* 16: 733-752.
- Lloyd, D. J., and Shore, A.
1938 The chemistry of the proteins (2nd ed.). Blakiston's Sons and Co., Inc., Philadelphia.
- Markley, M. C.
1938 The colloidal behavior of flour doughs. III. Studies upon the properties of flour-starch-water systems. *Cereal Chem.* 15: 438-444.
- Schmidt, C. L. A. (ed.)
1938 The chemistry of the amino acids and proteins. C. C. Thomas, Springfield, Ill.
- Shellenberger, J. A., and Libby, J. J.
1940 A physical dough-testing method applied to flour mill stream analysis. *Milling Production, Northwestern Miller* 5: 5, 6.
- Swanson, C. O.
1925 A theory of colloidal behavior in dough. *Cereal Chem.* 2: 265-275.
1937 Wheat and flour quality. Chapters 23-26. Burgess Pub. Co., Minneapolis.
1939 Variations in dough-development curves. *Cereal Chem.* 16: 625-643.
1941 Factors which influence the physical properties of dough. III. Effect of protein content and absorption on the pattern of curves made on the recording dough mixer. *Cereal Chem.* 18: 615-627.
- Wrinch, D. M.
1937 The cyclol hypothesis and the globular proteins. *Proc. Royal Soc., London. Series A, No. 907.* 161: 505-524.

THIAMIN RETENTION IN SELF-RISING FLOUR BISCUITS

R. A. BARACKMAN

Victor Chemical Works, Chicago, Illinois

(Received for publication November 3, 1941)

Published information on thiamin retention in "baking powder" products is so scarce that systematic investigation is justified. Copping and Roscoe (1937) found no demonstrable amount of vitamin B₁ remaining in bread, as indicated by the rat-growth method, when baking powder was used in place of yeast. Their "baking powder" bread consisted of flour mixed with 1.25% salt, 1.51% calcium acid phosphate, and 1.07% sodium bicarbonate. The dough was baked at once for 45 minutes at 450°–480°F. They cited the fact that Kent-Jones reported a similar dough as having a pH of 6.7–7.2, whereas yeast dough registered 5.5–5.9. Copping and Roscoe attributed the destruction of vitamin B₁ to its exposure to a mildly alkaline medium at a temperature of 100°C for a relatively long time.

It is well known that thiamin shows considerable resistance to heat in an acid or slightly acid medium. Exposure to temperatures of 100°C in these conditions causes little, if any, loss. However, in an alkaline medium there is extensive destruction even at low temperatures. As self-rising flours produce biscuits slightly on the alkaline side of neutrality, and baking time is relatively short, it might be expected that destruction of thiamin would be relatively small. The work reported here is a study of factors which affect the survival of thiamin in self-rising flour biscuits. Experiments were limited to the use of one flour. In order that they might be applicable to any flour, the tests involved the determination of the amount of added synthetic vitamin, thiamin hydrochloride, which is recovered in the baked biscuit irrespective of native thiamin. The thiochrome method of assay for thiamin was used throughout.

Methods

Self-rising flour and biscuits were prepared by the procedures outlined in Cereal Laboratory Methods (1941) for testing self-rising flour except where otherwise noted. The flour was a commercially milled, bleached product of about 60% extraction. To a weighed portion of self-rising flour was added 12% (flour basis) of hydrogenated shortening, which was mixed in by hand. Water or milk was added and the dough was mixed by machine. It was rolled out, cut, and panned as

TABLE I
 REPLICATE ASSAY ON SELF-RISING FLOUR BISCUITS IN THE pH RANGE FOUND IN COMMERCIAL SAMPLES
 (Milk used as the liquid; each gram of crumb except the blank received 3.9 μ g of added thiamin.)

Nep- tualizing value	Self-rising flour composition			Total B ₁ by assay			Total B ₁ less B ₁ of control bake			Recovery of added thiamin			Average recovery of thiamin %	pH of crumb
	Soda %	Anhyd. phos. %	Salt %	Test 1 μ g/g	Test 2 μ g/g	Test 3 μ g/g	Test 1 μ g/g	Test 2 μ g/g	Test 3 μ g/g	Test 1 %	Test 2 %	Test 3 %		
90	1.545	1.705	2.0	3.46	3.48	3.35	2.99	2.76	2.84	77	71	73	74	7.4
85	1.495	1.755	2.0	3.59	3.75	3.66	3.12	3.13	3.15	80	79	80	80	7.2
80	1.445	1.805	2.0	3.66	4.02	4.11	3.19	3.40	3.60	82	87	92.5	87	7.0
71.5	1.355	1.895	2.0	3.90	4.34	4.09	3.43	3.72	3.58	88	95	92	92	6.8
80	1.445	1.805	2.0	0.47	0.62	0.51	—	—	—	—	—	—	—	7.0

specified except that the dough pieces were placed in contact with each other in a round pan. Baking was done on the rotary shelf of an electric oven at 450°F; water biscuits were baked for 15 minutes, milk biscuits for 12 minutes. Upon removal, the biscuits were cooled, broken into small pieces, air-dried to constant weight, and ground through a food chopper.

Thiamin was determined on the air-dried, ground biscuits by the thiochrome method (Cereal Laboratory Methods, 1941) with modifications as suggested by the Food and Nutrition Committee of the National Research Council (unpublished).

Experimental

It was found early in this work that the same recovery values were obtained when the synthetic vitamin was blended with the flour as when it was dissolved in a portion of the liquid of the dough. As error might occur due to dusting if thiamin were blended into the flour, it was added as a solution. In all tests reported here 2.16 mg of thiamin per pound of flour was added. In each group of bakings, biscuits receiving no thiamin were included and they were assayed for native thiamin, this value being subtracted from that of biscuits receiving thiamin. The percentage recovery of the added thiamin was then calculated by use of records on the weight of ingredients, the weight of cut dough pieces before baking, and the weight of the baked and air-dried biscuits.

In preliminary tests the following variations in baking procedure were found not to affect the recovery of thiamin:

Machine vs. hand mixing of doughs

Single rolling vs. one or two foldings with subsequent rollings

Immediate baking vs. benching of doughs for $\frac{1}{2}$ hour before preparing for the oven

A round pan with doughs touching vs. an aluminum sheet with doughs spaced

Rotary shelf vs. a wire rack for support of pans in oven

Electric oven vs. gas oven at equivalent temperatures and times of baking

Baking time varying from 9 minutes to 17 minutes, water doughs.

The size of the biscuit had some effect on thiamin recovery. Biscuits of normal size made by rolling a dough out between supporting strips $\frac{3}{8}$ -inch high resulted in 88% recovery, whereas only 79% thiamin was recovered when $\frac{1}{4}$ -inch strips were used to obtain thin, crusty biscuits. The $\frac{3}{8}$ -inch strips were used in all tests herein reported.

Assay for thiamin was made with a representative sample of the ground, air-dried biscuit in all cases. However, in one test the crust assayed 2.46, whereas the crumb when plucked out assayed 3.10 μ g of thiamin per gram of air-dried product. In another case biscuits baked so as to have deeply browned, partially burned crusts showed decreased thiamin recovery. Overbaking was avoided in the reported tests.

The recovery of thiamin in biscuits made from self-rising flours containing varying ratios of soda to phosphate was studied. Table I presents replicate assays on self-rising flour biscuits in which the ratio of soda to anhydrous calcium acid phosphate was the variable. Each assay value represents triplicate determinations. The average of the standard deviations of replicated determinations was 3(%), which indicates average recoveries to be true values. The amount of thiamin recovered was found to be in proportion to the acidity of the biscuits within the range of pH studied. Table II gives additional evidence that the amount of thiamin surviving the baking process is dependent on the pH. In this table, however, the biscuits were made with water as the liquid, so that pH values and thiamin retention in the biscuits made with anhydrous phosphate are slightly lower than those shown in Table I, where milk was the liquid. The two phosphates were commercial products, the anhydrous calcium acid phosphate having a titration of 86.0 and the calcium acid phosphate monohydrate, 83.6. The ratio of soda to phosphate was varied as before, but the total of soda plus phosphate was held constant in order to prevent any appreciable variation in the amounts of total residual salts.

TABLE II

EFFECT OF ACIDITY AND ALKALINITY ON THIAMIN RECOVERY IN
SELF-RISING FLOUR BISCUITS MADE WITH WATER

Neutralizing value	Anhydrous calcium acid phosphate + soda = 3% of flour weight		Calcium acid phosphate monohydrate + soda = 3.625% of flour weight	
	Biscuit pH	Thiamin recovery	Biscuit pH	Thiamin recovery
100	8.3	% 49	8.4	% 28
90	7.7	76	7.7	60
80	7.3	81	7.3	67
70	7.0	85	7.0	82
60	6.7	91	6.7	80

Table II indicates that optimum thiamin recovery is attained in biscuits that are neutral or slightly acid. The data also show greater thiamin retention at the same pH level in the anhydrous phosphate series. It was thought that greater destruction may have occurred in the monohydrate series during the baking of the biscuits. A test was made wherein biscuits were removed from the oven at consecutive time intervals and the pH of the crumb was obtained after cooling. It was found that a maximum in pH values occurring after 4 to 5 minutes of baking is followed by a rapid drop after 7 to 8 minutes to slightly above the final pH. The time at which the maximum pH occurs is of brief

duration and coincides with the attainment of a temperature of 85°–95°C within the biscuit.¹

It will be observed in Table III, representing water biscuits, that phosphate of the monohydrate type causes a distinctly alkaline condition to exist temporarily during baking, whereas the anhydrous phosphate causes only a slightly alkaline condition.

TABLE III
MAXIMUM pH ATTAINED IN BISCUITS DURING THE BAKING
OF SELF-RISING FLOUR DOUGHS

Self-rising flour formula			Liquid used in baking	Maximum pH during baking	pH of fully baked biscuit crumb
Soda	Phosphate	Salt			
%	% (Anhydrous)	%			
1.25	1.5	2.0	Water	7.5	7.3
1.25	1.5	2.0	Milk	7.35	7.2
1.25 ¹	1.75	2.0	Milk	7.1	6.9
	(Monohydrate)				
1.5	1.875	1.75	Water	8.25	7.25
1.5	1.875	1.75	Milk	7.75	7.2
1.5 ¹	2.125	1.75	Milk	7.3	7.0

¹ Enriched self-rising flour formulas recommended.

Table IV supplies thiamin recovery values with biscuits baked under like conditions. It may be concluded that reactions of the anhydrous phosphate with soda within the baking biscuit are more favorable to the retention of thiamin. The use of milk in place of water reduced the maximum pH attained with both types of phosphates and materially improved thiamin retention. The most favorable conditions existed in milk biscuits made from self-rising flour, as proposed for enrichment purposes.

The formula for the commercial manufacture of enriched self-rising flour should be such that an optimum retention of thiamin will be obtained in the baked biscuits. It would not be practical commercially to mix self-rising flours so that a pH of say 6.5 would be obtained in those made with water as this acidity would tend to result in a slightly metallic acid taste and the cost would be excessive. A pH of 7.1 in biscuits made with water seemed a suitable value. Table IV presents comparative results of bakings made with the present standard formulas and by enriched flour formulas proposed for self-rising flours. Both water and milk biscuits were baked in duplicate twice; the average recovery values represent four separate bakings with triplicate assay on

¹ The temperature attained during the baking of biscuits was previously reported from these laboratories (1938, Cereal Chem 15: 843–45). As bakings were conducted under like conditions of technique, oven temperature, and type of oven, the data there reported may be applied here.

TABLE IV
THIAMIN RECOVERY IN BISCUITS MADE FROM THE PROPOSED ENRICHED SELF-RISING FLOUR FORMULAS
AND THE PRESENT STANDARD COMMERCIAL FORMULA
(Each test represents triplicate assays on duplicate bakings)

Formulas	Soda	Calcium acid phosphate	Salt	Liquid	Test 1		Test 2		Average thiamin percentage
					pH	Recovery	pH	Recovery	
Standard Standard	% 1.25 1.25	% Anhydrous 1.5 Anhydrous 1.5	% 2.0 2.0	Water Milk	7.4 7.3	% 76 78	7.3 7.1	% 78 84	% 77 81
Enriched Enriched	1.25 1.25	Anhydrous 1.75 Anhydrous 1.75	2.0 2.0	Water Milk	7.0 7.0	87 86	7.1 7.0	77 83	82 84.5
Standard Standard	1.5 1.5	Monohydrate 1.875 Monohydrate 1.875	1.75 1.75	Water Milk	7.3 7.1	69 79	7.25 7.1	73 80	71 80
Enriched Enriched	1.5 ^a 1.5	Monohydrate 2.125 Monohydrate 2.125	1.75 1.75	Water Milk	7.0 6.9	79 89	7.0 6.9	75 80	77 84.5

each set. It will be noted that milk biscuits resulted in pH values 0.2 unit lower than water biscuits and retained an average of 6% more thiamin. There was less spread in thiamin recovery between water and milk biscuits when the self-rising flour contained the anhydrous phosphate rather than the monohydrate. The proposed self-rising flour formulas in which 0.25% additional calcium acid phosphate is included with the present standard formulas resulted in thiamin recovery of 85% when biscuits were made with milk as the liquid.

Discussion

The thiamin value of enriched self-rising flour biscuits when baked under conditions favorable to the optimum preservation of thiamin may be approximated. For practical purposes 80% to 85% of the added thiamin may be used for calculating thiamin in biscuits. The loss of 15% to 20% of the 2.16 mg added per pound of flour, or 0.32 mg, represents approximately the amount of native thiamin in flour, so that the original amount of thiamin added is the amount in the final biscuit.

Thiamin recovery may vary somewhat with the type of baked product, but it appears that optimum retention will be attained if the pH does not become greater than 7.1-7.2 during the baking process. It will be of interest to compare the results reported here with those obtained with other self-rising flour products.

It may be predicted from this work that the use of phosphated flour instead of plain flour will improve greatly the retention of thiamin in biscuits made with baking powders as most baking powders result in biscuits considerably above 7.0 pH.

Summary

The recovery of thiamin added to self-rising flour doughs is shown to be in proportion to the acidity of the biscuits; the lower the pH, the better the recovery. About 85% of the thiamin added survives the baking process if the pH of the baked product is 7.1 or lower.

Commercial self-rising flour formulas, if revised so as to include 0.25% additional calcium acid phosphate in the formula for enriched self-rising flour, would result in optimum retention of thiamin in the finished biscuits.

The use in self-rising flour of calcium acid phosphate of the anhydrous type results in slightly less loss of thiamin during the baking process than the use of the monohydrate. Biscuits made with milk show better thiamin retention than those made with water. Baking conditions and methods of dough preparation have no appreciable effect on thiamin recovery provided a thin, crusty biscuit is not made.

Acknowledgment

The author is indebted to Mr. A. R. Wreath, who performed all assays for thiamin by the thiochrome method.

Literature Cited

- American Association of Cereal Chemists
1941 Cereal laboratory methods (4th ed.). American Association of Cereal Chemists, Lincoln, Nebraska.
Copping, A. M., and Roscoe, M. H.
1937 The water-soluble B-vitamins in yeast, flour, and bread. *Biochem. J.* 31: 1879-1902.

A STUDY OF THE NET WEIGHT CHANGES AND MOISTURE CONTENT OF WHEAT FLOUR AT VARIOUS RELATIVE HUMIDITIES¹

C. A. ANKER and W. F. GEDDES, with C. H. BAILEY

Division of Agricultural Biochemistry, Minnesota Agricultural Experiment Station, University Farm, St. Paul, Minnesota

(Received for publication October 7, 1941)

The significance of the moisture content of wheat and wheat products in relation to keeping quality and to changes in weight during storage has stimulated many studies of the changes in moisture content and net weight of such products under different conditions of storage. The majority of the experiments on flour reported in the literature—Willard (1911); Guthrie and Norris (1912); Sanderson (1914, 1915); Swanson, Willard, and Fitz (1915); Stockham (1917); Herman and Hall (1921); Frank and Campbell (1922); Arpin and Pecaud (1923); Frank (1923); Smith and Mitchell (1925); Fairbrother (1929); Thiessen (1933); Krtinsky (1937); Cathcart and Killen (1939), and others—have dealt with variations in net weight and moisture content when flours were exposed to varying influences of the atmosphere as encountered under natural storage conditions for different periods of time. These studies have clearly established that flour, in common with wheat and other cereal products, is hygroscopic, its moisture content fluctuating with changes in the relative humidity of the surrounding atmosphere. Because of its more finely divided state and the method of handling, flour responds more readily to changes in atmospheric humidity than does bulk grain. Small packages exhibit greater and more rapid changes in net weight and moisture content than larger ones when stored under the same conditions. In regard to the influence of the container, Krtinsky (1937) found a greater rate of moisture loss in jute than in paper bags

¹ Paper No. 1930, Scientific Journal Series, Minnesota Agricultural Experiment Station.

but Cathcart and Killen (1939) in studies on flour stored in paper-lined, jute, grain, and cotton bags found that the kind of sack had little effect on the variation in moisture content.

Frank (1923) found that while there was no appreciable difference in the rate of increase in the weight of moisture-free hard and soft winter wheat flours, hard winter wheat flour of normal moisture content retained more moisture than soft winter wheat flour exposed to a dry atmosphere.

In general, the experiments under uncontrolled storage conditions indicate that flour as normally packed will lose weight unless the relative humidity of the atmosphere is in the region of 60% or more. Moreover, the experiments of Fairbrother (1929) have shown that flour subjected to low relative humidities absorbs moisture slowly on re-exposure to high humidity and does not recover all the moisture lost; for example, a flour sample after re-exposure to an original relative humidity of 75% for 17 days failed to recover the weight lost during one night at 40% relative humidity. Fairbrother pointed out that, even at normal temperatures, the drying of flour apparently permanently reduces its hydration capacity. In this connection, Smith and Mitchell (1925) found that flour samples which were oven-dried after exposure to the laboratory atmosphere for 42 hours failed to regain all the weight lost during the drying period upon re-exposure to the laboratory atmosphere.

A few investigators—Bailey (1920); Fairbrother (1929); and Anderson (1937)—have determined the hygroscopic equilibria of flour in atmospheres of controlled relative humidity. Bailey found that the moisture content of patent flour exposed to atmospheres of 30, 50, 70 and 80% relative humidity at 25°C (77°F) contained approximately 5.2, 8.0, 12.1, and 15.0% moisture respectively at equilibrium, the curve representing this relation being parabolic in shape. These results were obtained by exposing 5-gram flour samples in aluminum moisture dishes over aqueous sulfuric acid solutions of the required concentration to provide the desired humidity levels at 25°C and determining the moisture content of the flour, after equilibrium had been attained, by a vacuum oven procedure.

In Fairbrother's (1929) controlled humidity experiments, conducted by exposing flour of 13.1% moisture content (method not stated) at approximately 25°C to air in contact with definite concentrations of sulfuric acid and calculating the equilibrium moisture from the change in weight, a linear relation was found between hygroscopic moisture and relative humidities varying between 15% and 90%. The equilibrium moistures obtained by Fairbrother were much higher than those secured by Bailey; for relative humidities of 30, 50, 70 and 80% Fairbrother found the

hygroscopic moisture to be approximately 9.4, 10.7, 13.8 and 15.5% respectively. Anderson (1937) determined the equilibrium relative humidity of hard winter wheat and wheat stocks by placing samples of varying moisture content in closed containers and reading the relative humidity of the atmosphere over the samples with a Crova hygrometer after equilibrium had been established. His hygroscopic equilibria values for hard winter wheat flour cover the range between 50% and 75% relative humidity; at 50% and 75% relative humidity the equilibrium moistures were 12.9% and 15.4% respectively, which exceed those reported by Fairbrother.

From this brief survey of the literature it is clear that wheat flour is hygroscopic, the rate of change in net weight and moisture content being dependent upon the size of the package and the difference between the moisture content of the flour and the equilibrium moisture corresponding to the existing relative humidity under the storage conditions. There is, however, a distinct lack of agreement regarding the effect of different kinds of containers on the rate at which equilibrium is attained and, of even more importance, in the equilibrium values themselves. The discrepancy between the data reported by Bailey (1920), Fairbrother (1929), and Anderson (1937) may be due to differences in the hydration capacity of the flours used or in the technique employed in determining relative humidity and moisture content. Much of the data on changes in the moisture content of flour under natural storage conditions was obtained prior to the official adoption and widespread use of the vacuum oven and 130°C one-hour air-oven methods for determining the moisture content of cereal products.

These considerations prompted the present investigation, the object of which was to determine as carefully as possible the changes in net weight and moisture content of wheat flour packed in different sizes of paper and cotton sacks and stored under controlled humidity conditions until equilibrium was attained. The large-scale storage tests were supplemented by laboratory studies designed to determine the hygroscopic equilibria of wheat flour over a wider range of humidities than was practical in the large-scale tests and to ascertain the effects of temperature and previous history of the flour in regard to its moisture content on the hygroscopic equilibria values.

Experimental

In order that the results might be of the greatest practical significance an 83% patent flour, commercially milled from a blend of hard red spring and hard winter wheats for the family trade and normally sold in 5, 10, and 24½ pound paper and cotton sacks, was used throughout

the studies. The protein content of the flour was 10.8% and the ash content 0.40%, both expressed on a 13.5% moisture basis.

Large-scale storage tests: For the large-scale storage tests, two similar specially designed heavily insulated cabinets, each with a storage capacity of approximately 47.5 cubic feet and completely air-conditioned for temperature and relative humidity, were made available to us. The wet- and dry-bulb elements of an air-operated recording regulator—the air-output from which operated pneumatic-electric switches which actuated the various pieces of equipment necessary to condition the air to the desired value—were located at the entrance of an external duct which carried the air

TABLE I

DATA SHOWING ACCURACY OF TEMPERATURE AND HUMIDITY CONTROL
IN AIR-CONDITIONED STORAGE CABINETS

	Approximate conditions maintained in the cabinet			
	70°F 35% RH	70°F 45% RH	70°F 60% RH	70°F 75% RH
Minimum dry bulb, °F	70	69	70	60
Minimum wet bulb, °F	55	56	61	63
Maximum relative humidity, %	37	43	60	73
Maximum dry bulb, °F	72	71	72	71
Maximum wet bulb, °F	56	58.5	62	64
Minimum relative humidity, %	35	47	58	70
Mean dry bulb, °F	71	70	71	70
Mean relative humidity, %	36	45	59	72

from the cabinet to the conditioner. In order to assure uniform air distribution, the conditioned air was delivered to the cabinet through a distributing device located in the center of the cabinet ceiling. The wet- and dry-bulb thermometers were checked against a thermometer calibrated by the U. S. Bureau of Standards prior to the commencement of the trials.

The accuracy of the humidity control, as computed from a survey of the wet- and dry-bulb temperature records made during the actual storage trials, is indicated by the data recorded in Table I. The accuracy is greater than would at first be expected from the recorded fluctuations of wet- and dry-bulb readings which were fairly large, especially the former. This is due to the fact that, from observation, the minima and maxima of the wet- and dry-bulb readings occurred simultaneously; this is reasonable since an increase or decrease in the dry-bulb temperature also raises or lowers respectively the wet-bulb temperature. As a conservative general statement it may be said that the dry-bulb temperature was

held to within approximately $\pm 1^{\circ}\text{F}$ and the relative humidity to within $\pm 2\%$ to 3% .

It was originally planned to carry out storage trials at a temperature of 70°F (21.1°C) and relative humidities of 30, 45, 60, and 75% respectively; the actual storage conditions, as shown by the mean values recorded in Table I for the lowest and highest humidities, deviated considerably from those originally planned. In the instance of the lowest humidity, the cabinet was actually adjusted to maintain 30% relative humidity and the storage trials were begun; however, because of the limited capacity of the cooling system, it was necessary to readjust the cabinet to a higher relative humidity (36%). As only two cabinets were available the tests at 45% and 72% and those at 36% and 59% relative humidity respectively were made simultaneously.

For the storage tests 5-pound, 10-pound, and $24\frac{1}{2}$ -pound paper and cotton sacks were machine packed consecutively from the flour bin feeding the packing machinery. During the packing operation four flour samples were taken at intervals for determination of original moisture content. The cotton sacks were machine sewn, the 10- and $24\frac{1}{2}$ -pound paper sacks machine tied, and the 5-pound paper sacks glue-sealed.

For the trials at 45% and 72% relative humidity 16 paper and cotton sacks of each weight (5- and 10-pound) were packed, while 20 of each kind and size were employed for each of the remaining two humidity levels; half of the above samples were used for storage at each relative humidity. Owing to limitations in cabinet space, only two $24\frac{1}{2}$ -pound sacks (one paper and one cotton) were stored under each of the four storage conditions. The sacks were numbered consecutively and weighed to the nearest 0.5 gram on a Gurley precision balance (capacity 25 kg on each pan, sensitivity 60 mg at full load). The weights employed were adjusted, and certified by the Minnesota Department of Weights and Measures to fall within the allowable tolerances of the U. S. Bureau of Standards for "Class C Commercial Test Weights" (which are identical with the tolerances established for Class I technical weights in the range of from 1 to 500 grams).

After all the sacks were weighed, an operation which required approximately two hours, the $24\frac{1}{2}$ -pound sacks were placed horizontally on the bottom shelf of the storage cabinet. The 5- and 10-pound paper and cotton sacks were placed vertically on the remaining two shelves, their positions being randomized. This procedure not only completely randomized the sacks assigned to the two simultaneous storage conditions and their location within the respective cabinets, but also the order in which the original weights of the sacks assigned to the two cabinets were obtained. The sacks were weighed after three days' storage and

weekly thereafter for the duration of the trials. Each week one 5- and one 10-pound paper sack and one 5- and one 10-pound cotton sack for each storage condition were selected at random and retained for moisture determinations. Immediately after weighing, the contents of the selected sacks were placed in air-tight cans; later, the contents of each can were thoroughly mixed in a small MacLellan batch mixer and four samples removed and placed in air-tight containers. One sample was employed in this laboratory for moisture determinations in triplicate by both the vacuum oven and 130°C one-hour air-oven methods as described in *Cereal Laboratory Methods* (4th ed., 1941) published by the American Association of Cereal Chemists. As a check on our laboratory results, the remaining three samples were distributed to each of three commercial mill laboratories which determined and reported their moisture values in duplicate by the 130°C one-hour air-oven method.

TABLE II
MEAN EMPTY SACK WEIGHTS FOR THE VARIOUS STORAGE CONDITIONS

Kind of sack	Mean empty sack weight			
	36% RH	45% RH	59% RH	72% RH
	<i>g</i>	<i>g</i>	<i>g</i>	<i>g</i>
5-lb paper	20.0	20.5	20.5	21.0
5-lb cotton	17.0	17.0	17.0	17.0
10-lb paper	39.0	39.5	40.0	40.5
10-lb cotton	26.5	26.5	27.0	27.5
24½-lb paper	66.5	67.0	68.0	69.0
24½-lb cotton	50.0	50.0	51.0	52.0

In order to permit the calculation of the net weight of flour in each size and kind of container, twelve 5- and twelve 10-pound empty paper and cotton sacks and five 24½-pound paper and cotton sacks were placed in the storage cabinets maintained at each of the storage conditions and the weights determined after equilibrium was attained. The mean sack weights are recorded in Table II.

Laboratory studies of hygroscopic equilibria at constant temperature: Employing samples of the same flour used in the storage tests, four series of hygroscopic equilibrium determinations were made, each at 10% intervals, over the range from approximately 10% to 80% relative humidity. Three series were conducted at a constant temperature of 25°C (77°F) with the flour adjusted to initial moisture contents of 6.5%, 12.2%, and 14.7% respectively; the remaining series was made at 37°C (98.6°F) with flour at 12.2% moisture. The bulk portion of flour reserved for these studies was not stored in air-tight containers

and was found to contain 12.2% moisture at the time the samples were taken for the laboratory studies. The subsample of 6.5% moisture was secured by exposing a portion of the above sample in the thermostat at 25°C, while the sample at 14.7% moisture content was obtained by exposing another portion over water at a temperature of 2°C. Each of the subsamples was thoroughly mixed in a MacLellan batch mixer and reserved in air-tight containers.

The hygroscopicity was determined by exposing the flour over sulfuric acid solutions in closed vessels placed in an air thermostat equipped with a fan and maintained at the required temperature to within $\pm 0.1^\circ\text{C}$. In adjusting the atmospheres to the desired relative humidities, the vapor pressure data of Wilson (1921) for aqueous sulfuric acid solutions were employed.

In order that the flour samples might be weighed at intervals without removing them from the atmospheric conditions to which they were exposed and to lessen the possibility of errors due to creeping of the sulfuric acid solution, a technique similar to that described by Fisher (1927) in his studies of the rate of drying of wheat flour over concentrated sulfuric acid was employed. The humidity chambers consisted of battery jars approximately $5\frac{1}{2} \times 6\frac{3}{8} \times 10\frac{1}{2}$ inches high. The rim of each jar was ground and fitted with a plate-glass cover $5\frac{3}{4} \times 7$ inches through the center of which a $\frac{1}{4}$ -inch hole was drilled. An aluminum pan equipped with a stirrup was suspended from a nichrome wire passing through the hole of the cover plate, the free end of the wire being formed into a loop. Eight battery jars were placed in a row in the thermostat, the wires passing through small holes drilled in the top of the thermostat and the aluminum pan supported by inserting short glass rods through the loops. A balance from which the left pan was removed was mounted on a drawer track on top of the cabinet. Attached to the left stirrup was a fine nichrome wire which extended through a hole in the bottom of the balance and terminated in a small hook. The balance could thus be brought directly over each of the battery jars. In weighing, the hook was fastened to the loop of the wire supporting the aluminum pan and stirrup (upon which the moisture dishes were placed), and the glass rod removed. When weighing was not in progress, the holes in the top of the cabinet were sealed with small corks and those in the cover plates by two microscope slides each notched on the edge so that they would fit closely around the wire. Stop-cock grease was used to seal the glass joints, care being taken that no grease came in contact with the wire supporting the aluminum pan.

In determining the hygroscopic equilibria, one liter of the required sulfuric acid solution was placed in the battery jar, a 5-g sample of the

flour contained in a standard aluminum moisture dish placed on the supporting aluminum pan, and the jar sealed with the cover plate and microscope slides. Weighings were made at daily intervals until constant weight was attained; in the instance of the lowest humidities, this required approximately two weeks. At the end of each trial, the concentration of the sulfuric acid solution was obtained by determining the density with a Westphal chainomatic balance and the relative humidity computed. The final moisture content of the flour was calculated from a knowledge of the initial moisture content and the change in weight; this procedure was found to give values in excellent agreement with the results obtained by actual determinations of the final moisture contents at equilibrium.

Results of storage trials: In the storage trials irregularities occurred in the tests at 36% and 59% relative humidity. The studies conducted at 36% were commenced with the cabinet adjusted to a relative humidity of 30% but after three days' operation it was found that the capacity of the cooling coil was insufficient to maintain this level against the large quantities of water vapor being contributed by the flour. The initial moisture content of the flour was 13.5% (air-oven method) and from available data it appeared that this level was the approximate equilibrium value for 65% relative humidity. Since a storage cabinet operating at this humidity was temporarily available, the flour sacks were removed from the "30% cabinet," weighed, and transferred to the 65% cabinet in the hope that the weight losses occasioned by three days' storage would be regained, in approximately the same time interval. In the meantime the empty cabinet was readjusted to operate at 36% relative humidity.

The sacks were weighed at intervals over a period of 11 days, when it became obvious from the rate of increase in weight of the 5- and 10-pound sacks that they would not recover their original weight unless stored for a prolonged period, if indeed at all. The 24½-pound sacks, however, more than regained their original weight. Accordingly, one each of the 5- and 10-pound paper and cotton sacks was reserved for a moisture test, and the remainder transferred to the previous cabinet now adjusted to 36% relative humidity. The initial moisture contents of the 5- and 10-pound sacks stored at this humidity level were therefore not uniform and were, as will be shown later, considerably lower than those stored at the other humidity levels.

The irregularity at 59% relative humidity occurred between the eighth and ninth week of storage, three days prior to the weighings for the ninth week and continuing two days thereafter. During this five-day period the mechanism controlling the water sprays failed, with the result that the relative humidity dropped to a low of approximately 44%. The

TABLE III
MEAN PERCENTAGE CHANGE IN NET WEIGHT OF FLOUR STORED AT 70°-71°F
(21.1°-21.7°C) FOR VARYING TIMES IN 5-, 10-, AND 24½-POUND PAPER
AND COTTON SACKS AT DIFFERENT RELATIVE HUMIDITIES

Storage period days	Number of sacks ¹	Percentage change in net weight											
		5-lb sacks			10-lb sacks			5- and 10-lb sacks				24½-lb sacks	
		Paper	Cotton	Paper and cotton	Paper	Cotton	Paper and cotton	Paper	Cotton	Paper and cotton	Standard error ²	Paper	Cotton
		%	%	%	%	%	%	%	%	%	%	%	%
FLOUR STORED AT 36% RELATIVE HUMIDITY AND 71°F (21.7°C)													
3	9	-1.21	-1.48	-1.34	-1.19	-1.32	-1.25	-1.20	-1.40	-1.30	.082	-1.66	-1.92
7	9	-2.01	-2.40	-2.20	-1.95	-2.12	-2.03	-1.98	-2.25	-2.12	.075	-2.28	-2.66
14	8	-2.69	-2.94	-2.81	-2.63	-2.74	-2.69	-2.66	-2.84	-2.75	.086	-2.90	-3.32
21	7	-3.07	-3.23	-3.15	-3.03	-3.16	-3.10	-3.05	-3.20	-3.12	.077	-3.34	-3.75
28	6	-3.29	-3.35	-3.32	-3.27	-3.34	-3.31	-3.28	-3.35	-3.31	.078	-3.65	-4.01
35	5	-3.37	-3.38	-3.37	-3.39	-3.41	-3.40	-3.38	-3.40	-3.39	.083	-3.85	-4.16
42	4	-3.30	-3.23	-3.26	-3.31	-3.34	-3.32	-3.30	-3.28	-3.29	.096	-3.89	-4.15
49	3	-3.35	-3.32	-3.34	-3.39	-3.41	-3.40	-3.37	-3.36	-3.37	.058	-4.00	-4.22
56	2	-3.26	-3.20	-3.24	-3.34	-3.35	-3.35	-3.30	-3.28	-3.29	.092	-4.02	-4.19
63	1	-3.14	-3.23	-3.18	-3.32	-3.33	-3.32	-3.23	-3.28	-3.26	—	-3.98	-4.13
70	1	-3.25	-3.30	-3.28	-3.32	-3.39	-3.66	-3.28	-3.64	-3.46	—	-3.99	-4.12
FLOUR STORED AT 45% RELATIVE HUMIDITY AND 70°F (21.1°C)													
3	8	-0.82	-0.93	-0.87	-0.70	-0.76	-0.73	-0.76	-0.84	-0.80	.048	-0.51	-0.60
7	8	-1.25	-1.32	-1.28	-1.06	-1.19	-1.13	-1.16	-1.26	-1.21	.055	-0.82	-0.97
14	7	-1.59	-1.63	-1.61	-1.42	-1.52	-1.47	-1.50	-1.58	-1.54	.049	-1.14	-1.30
21	6	-1.75	-1.77	-1.76	-1.61	-1.70	-1.65	-1.68	-1.73	-1.71	.036	-1.36	-1.51
28	5	-1.84	-1.84	-1.84	-1.73	-1.77	-1.75	-1.78	-1.80	-1.79	.034	-1.51	-1.63
35	4	-1.92	-1.94	-1.93	-1.84	-1.93	-1.89	-1.88	-1.94	-1.91	.067	-1.64	-1.76
42	3	-2.04	-2.08	-2.06	-1.99	-2.00	-1.99	-2.02	-2.04	-2.03	.027	-1.82	-1.91
49	2	-2.16	-2.18	-2.17	-2.10	-2.10	-2.10	-2.13	-2.14	-2.14	.019	-1.94	-2.03
56	1	-2.14	-2.20	-2.17	-2.14	-2.08	-2.11	-2.14	-2.14	-2.14	—	-2.06	-2.10
63	1	-1.96	-2.02	-1.99	-1.95	-1.87	-1.91	-1.96	-1.94	-1.95	—	-1.97	-1.98
67	1	-1.94	-2.00	-1.97	-1.91	-1.86	-1.88	-1.92	-1.93	-1.93	—	-1.94	-1.95
FLOUR STORED AT 59% RELATIVE HUMIDITY AND 71°F (21.7°C)													
3	10	0.12	0.27	0.20	0.20	0.23	0.22	0.16	0.25	0.21	.028	0.18	0.21
7	10	0.19	0.33	0.26	0.25	0.31	0.28	0.22	0.32	0.27	.037	0.24	0.28
14	9	0.32	0.46	0.39	0.40	0.44	0.42	0.36	0.45	0.40	.028	—	—
21	8	0.19	0.28	0.23	0.30	0.31	0.30	0.24	0.29	0.27	.023	0.31	0.33
28	7	0.10	0.23	0.17	0.19	0.23	0.21	0.15	0.23	0.19	.028	0.25	0.25
35	6	-0.11	0.00	-0.06	0.03	0.04	0.03	-0.04	0.02	-0.01	.018	0.12	0.12
42	5	-0.26	-0.15	-0.20	-0.12	-0.12	-0.12	-0.19	-0.13	-0.16	.019	0.00	-0.02
49	4	-0.08	+0.06	-0.01	+0.03	+0.07	+0.05	-0.02	+0.06	+0.02	.022	0.08	0.10
56	3	-0.16	-0.08	-0.12	-0.04	-0.02	-0.03	-0.09	-0.05	-0.07	.020	0.02	0.03
63	2	-1.49	-1.74	-1.62	-1.25	-1.48	-1.36	-1.37	-1.61	-1.49	.051	-0.85	-1.01
70	1	-0.79	-0.68	-0.74	-0.62	-0.62	-0.62	-0.70	-0.65	-0.68	—	-0.50	-0.54
77	1	-0.66	-0.57	-0.62	-0.48	-0.47	-0.48	-0.57	-0.52	-0.54	—	-0.41	-0.43
FLOUR STORED AT 72% RELATIVE HUMIDITY AND 70°F (21.1°C)													
3	8	0.68	0.94	0.81	0.67	0.77	0.72	0.68	0.86	0.77	.045	0.45	0.57
7	8	1.08	1.34	1.21	1.04	1.17	1.10	1.06	1.25	1.16	.050	0.75	0.92
14	7	1.32	1.51	1.42	1.26	1.38	1.32	1.29	1.44	1.37	.041	1.00	1.14
21	6	1.46	1.63	1.54	1.40	1.52	1.46	1.43	1.57	1.50	.050	1.16	1.29
28	5	1.45	1.60	1.52	1.41	1.50	1.46	1.43	1.55	1.49	.051	1.22	1.31
35	4	1.43	1.60	1.52	1.44	1.52	1.48	1.43	1.56	1.50	.044	1.28	1.38
42	3	1.49	1.66	1.57	1.51	1.58	1.54	1.50	1.62	1.56	.057	1.32	1.42
49	2	1.64	1.78	1.71	1.69	1.73	1.71	1.66	1.76	1.71	.060	1.45	1.55
56	1	1.61	1.69	1.65	1.74	1.68	1.71	1.68	1.68	1.68	—	1.47	1.57
63	1	1.61	1.64	1.62	1.72	1.67	1.70	1.66	1.66	1.66	—	1.48	1.55
67	1	1.62	1.66	1.64	1.73	1.65	1.69	1.68	1.66	1.66	—	1.50	1.56

¹ The values given in this column represent the number of 5- and 10-lb paper and cotton sacks respectively which were weighed at each storage period. Only one 24½-lb sack of each kind was employed.

² The values given in this column represent the pooled standard errors (single determination) for all 5- and 10-lb sacks.

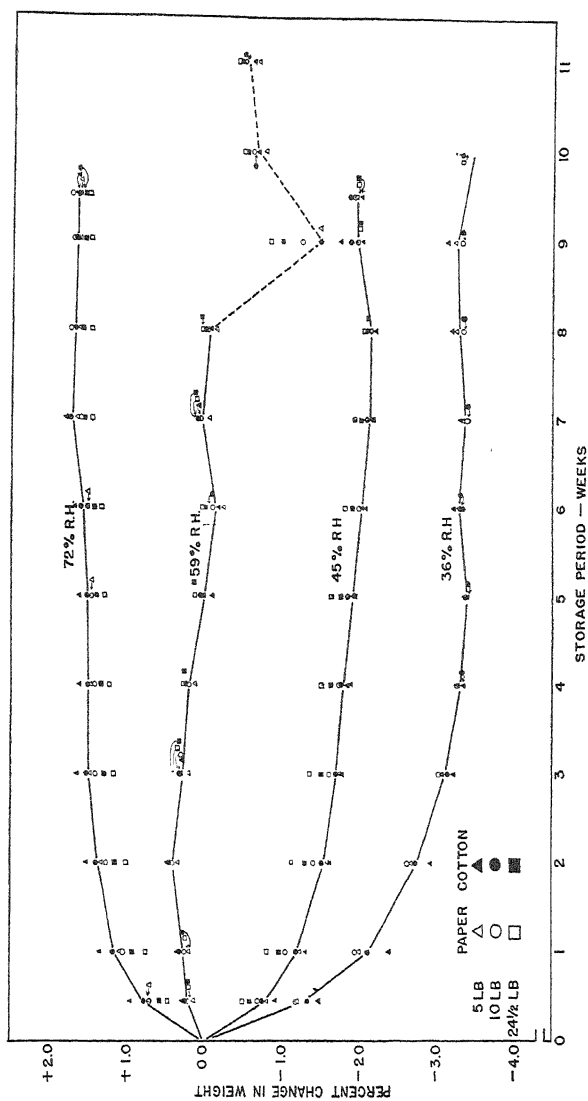


Fig. 1. Mean percentage changes in net weight of flour stored in 5-, 10-, and 24½-pound paper and cotton sacks at 70°-71°F (21.1°-21.7°C) and different relative humidities. (The lines drawn through the scatter of points represent the changes in the mean values for all 5-, and 10-pound sacks (paper and cotton) combined. In the storage tests at 59% relative humidity the humidifying apparatus failed three days prior to the weighings for the ninth week and continued for two days thereafter. During this five-day interval the relative humidity fluctuated around 44%.)

humidifying apparatus was repaired during this interval and the storage trials continued for a further two weeks.

The mean percentage changes in net weight of the flour stored in the various sacks for different intervals of time at each of the four humidity levels are recorded in Table III, together with the pooled standard errors (for single determinations) for all 5- and 10-pound sack weight changes at each interval of storage. The percentage changes in weight of the various sizes and kinds of sacks are represented graphically in Figure 1; the lines drawn through the scatter of points represent the mean values for the 5- and 10-pound paper and cotton sacks as recorded in Column 11 of Table III.

The mean results of triplicate determinations of flour moisture content for the various storage conditions are recorded in Tables IV and V.

TABLE IV

MOISTURE CONTENT OF FLOUR STORED AT 70°-71°F (21.1°-21.7°C) IN
5- AND 10-POUND PAPER AND COTTON SACKS FOR VARYING TIMES
AT DIFFERENT RELATIVE HUMIDITIES

Storage period	Moisture content ¹								
	5-lb sack			10-lb sack			5- and 10-lb sack		
	Paper	Cotton	Paper and cotton	Paper	Cotton	Paper and cotton	Paper	Cotton	Paper and cotton
days	%	%	%	%	%	%	%	%	%
FLOUR STORED AT 36% RELATIVE HUMIDITY AND 71°F (21.7°C)									
0	12.7	12.6	12.66	12.6	12.8	12.71	12.67	12.70	12.68
7	11.0	10.6	10.78	11.1	11.0	11.07	11.07	10.78	10.92
14	10.5	10.1	10.30	10.6	10.4	10.51	10.57	10.23	10.40
21	10.2	9.9	10.05	10.3	10.0	10.17	10.27	9.95	10.11
28	9.9	9.7	9.82	10.0	9.9	9.94	9.95	9.81	9.88
35	9.8	9.7	9.74	9.8	9.7	9.75	9.81	9.69	9.75
42	9.9	9.9	9.89	10.0	9.9	9.94	9.94	9.89	9.91
49	9.9	9.9	9.86	9.9	9.8	9.89	9.89	9.86	9.87
56	9.9	9.8	9.85	9.9	9.8	9.85	9.90	9.79	9.85
70	9.8	10.0	9.90	9.8	9.8	9.80	9.82	9.88	9.85
FLOUR STORED AT 45% RELATIVE HUMIDITY AND 70°F (21.1°C)									
0	13.3	13.3	13.30	13.3	13.3	13.30	13.30	13.30	13.30
7	12.2	12.0	12.11	12.3	12.1	12.17	12.22	12.05	12.14
14	11.8	11.7	11.78	11.8	11.8	11.83	11.82	11.79	11.80
21	11.7	11.6	11.66	11.8	11.6	11.68	11.75	11.59	11.67
28	11.6	11.5	11.56	11.6	11.5	11.56	11.63	11.49	11.56
35	11.6	11.5	11.53	11.6	11.5	11.58	11.61	11.50	11.55
42	11.4	11.2	11.30	11.3	11.3	11.27	11.31	11.26	11.28
49	11.3	11.3	11.27	11.4	11.3	11.35	11.33	11.29	11.31
67	11.5	11.4	11.43	11.4	11.4	11.42	11.45	11.39	11.42

¹ Air-oven method.

TABLE IV—*Continued*

Storage period days	Moisture content ¹								
	5-lb sack			10-lb sack			5- and 10-lb sack		
	Paper	Cotton	Paper and cotton	Paper	Cotton	Paper and cotton	Paper	Cotton	Paper and cotton
	%	%	%	%	%	%	%	%	%
FLOUR STORED AT 59% RELATIVE HUMIDITY AND 71°F (21.7°C)									
0	13.4	13.4	13.40	13.4	13.4	13.40	13.40	13.40	13.40
7	13.4	13.4	13.41	13.5	13.5	13.48	13.46	13.43	13.44
14	13.5	13.6	13.52	13.5	13.6	13.53	13.48	13.57	13.53
21	13.6	13.6	13.56	13.6	13.6	13.61	13.59	13.58	13.59
28	13.4	13.4	13.43	13.4	13.4	13.44	13.43	13.44	13.43
35	13.4	13.4	13.38	13.3	13.3	13.34	13.36	13.36	13.36
42	13.1	13.2	13.14	13.1	13.1	13.10	13.11	13.12	13.12
49	13.2	13.3	13.24	13.2	13.2	13.24	13.22	13.26	13.24
56	13.2	13.2	13.23	13.2	13.2	13.21	13.22	13.22	13.22
63	12.2	11.8	12.01	12.2	11.9	12.08	12.20	11.89	12.05
77	12.7	12.7	12.70	12.7	12.7	12.70	—	—	—
FLOUR STORED AT 72% RELATIVE HUMIDITY AND 70°F (21.1°C)									
0	13.3	13.3	13.30	13.3	13.3	13.30	13.30	13.30	13.30
7	13.9	14.1	14.01	13.8	13.9	13.86	13.86	14.01	13.93
14	14.1	14.2	14.19	14.1	14.2	14.15	14.12	14.21	14.17
21	14.4	14.4	14.42	14.2	14.3	14.27	14.32	14.37	14.34
28	14.3	14.3	14.31	14.2	14.3	14.24	14.27	14.27	14.27
35	14.3	14.4	14.38	14.4	14.4	14.36	14.35	14.39	14.37
42	14.4	14.5	14.43	14.3	14.2	14.26	14.33	14.36	14.34
49	14.5	14.5	14.50	14.4	14.6	14.48	14.43	14.55	14.49
67	14.4	14.4	14.41	14.4	14.4	14.40	14.41	14.40	14.41

¹ Air-oven method

TABLE V

INITIAL AND FINAL MOISTURE CONTENT OF FLOUR STORED IN 24½-POUND PAPER AND COTTON SACKS AT 70°–71°F (21.1°–21.7°C) AND DIFFERENT RELATIVE HUMIDITIES

Time of storage days	Storage conditions			Flour moisture content ¹			
	Temperature		Relative humidity	Initial		Final	
	°F	°C		Paper	Cotton	Paper	Cotton
70	71	21.7	36	13.7	13.7	9.9	9.7
67	70	21.1	45	13.3	13.3	11.4	11.4
77	71	21.7	59	13.4	13.4	12.8	12.8
67	70	21.1	72	13.3	13.3	14.2	14.2

¹ Air-oven method.

While moistures were determined in this laboratory by both the vacuum-oven and air-oven methods and in three commercial laboratories, the results were in such excellent general agreement that only the mean air-oven values obtained in this laboratory are presented. For all samples, the air-oven values determined by this laboratory averaged 0.058% lower than for the vacuum-oven method; the experimental errors were virtually identical, the standard error of a single determination being 0.042% for the air-oven and 0.041% for the vacuum-oven method. In the instance of 131 samples, for which strictly comparable air-oven techniques were employed in all four laboratories, the mean moisture obtained by us was 12.163%, and the general mean for the three commercial laboratories was 12.115%. In view of this excellent agreement, it is immaterial, in relation to the general conclusions, as to which moisture data are employed. However, because of its rapidity and convenience the air-oven method is in more general use than the vacuum-oven method in mill laboratories and the results by this procedure were arbitrarily selected for presentation.

With reference to the percentage changes in net weight shown in Table III and Figure 1, it must be recalled that the initial moisture content of the flour stored at 36% relative humidity was not uniform because of the differential response of the various kinds of sacks to the preliminary exposures at 30% and 65% relative humidity. As shown in Tables IV and V, the initial moisture content of the 24½-pound sacks stored at 36% relative humidity was 13.7%, whereas the moisture contents of the 5- and 10-pound sacks ranged between 12.6% and 12.8%. For this reason the data for the 24½-pound sacks have been omitted from Figure 1. The mean initial moisture content of the flour as packed for the storage trials at 45% and 72% relative humidity was 13.3%; for the sacks stored at 59% the mean moisture was 13.4%. These means are based on triplicate determinations made on four samples taken at intervals during packing. Variance analyses revealed that the differences between the moisture contents of the four samples taken during each packing, respectively, were not significant. It can therefore be assumed that the moisture contents for all sacks packed at any one time were identical.

These studies of the percentage changes in weight show that flour responds very readily to changes in relative humidity. The rate of change is relatively rapid during the first few days of storage, the actual rate for any one type of container depending upon the magnitude of the difference between the existing relative humidity and the equilibrium humidity corresponding to the moisture content of the flour.

The initial response to any one set of storage conditions depends upon the size and kind of package. Thus, from Table III and Figure 1

it will be noted that the rate of change in weight decreases with increasing package size and is greater for cotton than for paper sacks; as the storage period is prolonged the cumulative percentage changes in weight for the respective containers tend to equalize. This differential initial response due to the size and kind of sack is most pronounced in the storage trials at 36, 45, and 72% relative humidity where the rate of change is relatively rapid. At 59% relative humidity, the mean rate of change is slow since this humidity level is close to the equilibrium humidity corresponding to the initial moisture content of the flour stored in the 5- and 10-pound sacks. That the differences discussed above are statistically significant has been shown by variance analyses of the data for the 5- and 10-pound sacks for the early storage periods at the different humidity levels; for these periods there was sufficient replication to provide a precise test. The significance of the differences in change in weight of the 5- and 10-pound sacks may also be ascertained by computation from the standard errors for single determinations given in Table III.

The rapidity of the change in weight with changing storage conditions and the differential response of the various sizes and kinds of sacks is perhaps most clearly shown by the data obtained after the failure of the humidity control between the eighth and ninth week of storage at 59% relative humidity. This failure occurred three days before the weighings for the ninth week, the relative humidity rapidly dropping to approximately 44% and fluctuating around this level for five days when the repair was completed. At the eighth week the percentage changes in weights for the different sacks were similar, varying only from +0.02 to -0.16%. Upon three days' storage at the lower humidity, the 5-pound cotton sack had decreased 1.74% in weight, the 5-pound paper sack 1.49%, the 10-pound cotton 1.48%, the 10-pound paper 1.25%, the 24½-pound cotton 1.01% and the 24½-pound paper 0.85%. The average cumulative percentage weight change for the 5- and 10-pound paper and cotton sacks was 1.49% or a decrease of 1.42% between the eighth and ninth week. Despite the fact that the repair was completed two days after the weighings for the ninth week, and the sacks re-exposed to 59% relative humidity five days (the same period of time for which they were accidentally exposed to the lower humidity) before the weighings for the tenth week, the average cumulative weight change for the 5- and 10-pound paper and cotton sacks was -0.68%, a regain of only about 55% of the weight lost. After a further week's storage, there was an additional average regain of only 0.14%. The rate of loss in weight was therefore greater than the rate of regain and the data indicate that the original moisture content would not be reached

except after prolonged storage, if indeed at all. It is of interest to note that the average moisture content for the 5- and 10-pound sacks at the ninth week of storage at 59% relative humidity was 12.05% as compared with 13.22% at the eighth week; thus, the drying of the flour to a 12.0% moisture level apparently altered its hygroscopicity more or less permanently.

The irregularity already mentioned in connection with the storage tests at 36% relative humidity confirms these observations. The weight lost by the 5- and 10-pound sacks in four days at 30% relative humidity was not regained in 11 days' storage at 65%. The original moisture content for all the sacks was 13.4%; upon exposure to 30% and then 65% relative humidity the average moisture content for the 5- and 10-pound sacks was 12.7% and that for the 24½-pound sacks 13.7%. The 24½-pound sacks more than regained their original weight. The reason for this discrepancy in behavior is not clear. While the differential response of the sacks to exposure at 30% relative humidity carried the moisture content of the flour stored in the 5- and 10-pound sacks to a moisture content averaging approximately 0.5% lower than was the case for the 24½-pound sacks, this variation does not appear sufficient to account for the difference in behavior unless the hydration capacity of flour is greatly influenced by relatively slight reductions in moisture content.

Examination of the data given in Table III and Figure 1 does not indicate that any well established equilibrium in net weight has been attained under any of the storage conditions. The behavior of the flour stored at 59% and 72% relative humidity is erratic, particularly at the former level. Careful examination of the wet- and dry-bulb temperature records failed to reveal any trend in the temperature or relative humidity with time of storage which would explain the drifts in the percentage weight changes. Since temperature was held to within 1°F and relative humidity to within 2% to 3%, and the work was carefully performed, it must be concluded that flour is extremely sensitive to slight changes in storage conditions and/or that the hydration capacity of flour varies with time and, accordingly, has no precise equilibrium value even under fixed storage conditions.

The moisture data recorded in Table IV for the 5- and 10-pound sacks follow the same general trends as the percentage changes in weight given in Table III. In fact, the moisture content at each storage period may be calculated from a knowledge of the initial moisture content, the initial net weight, and the corresponding weights at each storage period. These computations have been made and the calculated and determined moisture values found to be in good agreement, the maximum discrepancy being 0.3%.

The moisture content of flour packed in 5- and 10-pound sacks changes quite rapidly when stored at relative humidities which differ appreciably from the equilibrium humidity corresponding to the moisture content of the flour, the rate of change decreasing as the moisture content approaches the equilibrium value. For example, during the first week of storage at 36% relative humidity, the moisture content of the flour fell from an initial mean value of 12.68% to 10.92% or a mean loss of 1.76%; in the same interval of time flour containing initially 13.3% moisture lost an average of 1.16% moisture when stored at 45% humidity while it gained 0.63% moisture upon storage at 72% relative humidity. The rate of change in moisture content during the first few

TABLE VI
HYGROSCOPIC EQUILIBRIA OF FLOUR OF VARYING INITIAL MOISTURE CONTENTS
AT DIFFERENT RELATIVE HUMIDITIES AND CONSTANT TEMPERATURES¹

Temperature 25°C						Temperature 37°C	
Initial flour moisture 6.5%		Initial flour moisture 12.2%		Initial flour moisture 14.7%		Initial flour moisture 12.2%	
RH ²	EM	RH	EM	RH	EM	RH	EM
%	%	%	%	%	%	%	%
10.6	6.1	10.6	5.9	10.9	6.0	12.0	5.5
20.4	7.5	20.6	8.2	20.8	8.1	22.8	7.3
29.6	8.7	29.6	9.5	29.2	9.3	31.0	8.6
39.2	10.1	39.2	11.0	39.1	10.9	40.9	10.1
49.9	11.5	50.0	12.3	49.4	11.9	51.4	11.3
59.2	12.8	59.5	13.2	59.0	13.5	60.5	12.4
68.7	14.1	68.7	14.3	70.4	14.0	69.2	13.6
78.7	15.9	78.8	16.1	78.4	15.8	78.8	15.4

¹ All moisture determinations were carried out by the air-oven method.

² RH = relative humidity. EM = equilibrium moisture.

weeks of storage at each of these humidity levels increases with a decrease in the size of the container and is greater for cotton than for paper sacks. In the storage trials at 59% relative humidity, there is no significant differential response due to the size or kind of sack since this humidity level is close to the equilibrium humidity corresponding to the moisture content of the flour, and the rate of change is relatively slow. Statistical analyses of the data for 45%, 59%, and 72% relative humidity confirmed the above observation.² For the trials at 45% and 72% humidity, significant interactions were found between storage period and size and kind of sack, and are a reflection of the fact that initially there is a wide differential response of these different sacks to the storage conditions, but as the storage progresses the moisture values tend to

² A statistical analysis was not conducted for the 36% humidity level since the moisture contents for the different sacks were not identical at the commencement of the storage period.

approach a common equilibrium independent of the nature or capacity of the sack. Insignificant interactions for size and kind of sack showed that the relative behavior of the flour stored in the 5- and 10-pound sacks is the same whether they are made of paper or cotton.

Results of laboratory determinations of hygroscopic equilibria: The mean results of duplicate determinations of the hygroscopic equilibria of the flour at initial moisture contents of 6.5%, 12.2%, and 14.7%

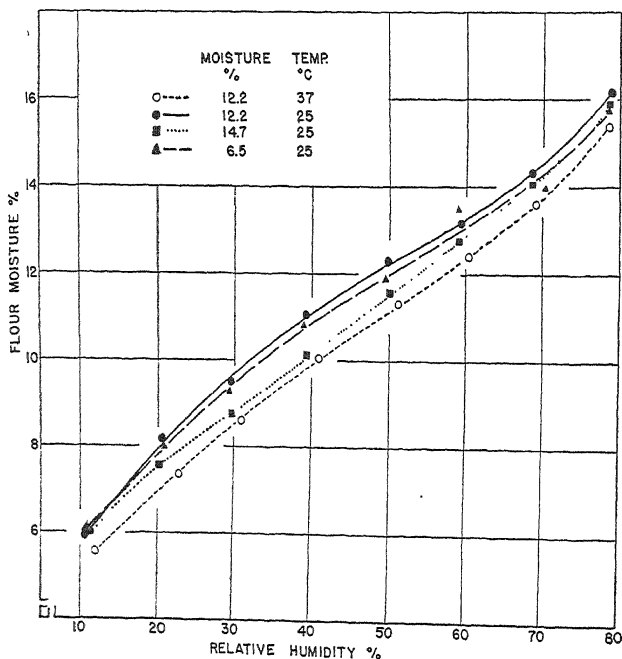


Fig. 2. Hygroscopic equilibria of flour at 25°C (77°F) and 37°C (98.6°F) conditioned to various initial moisture contents.

moisture at various relative humidities are recorded in Table VI and represented graphically in Figure 2. These results show that the hygroscopic equilibrium of a particular flour at any given humidity is not a constant value but depends on its original moisture content and the temperature at which the equilibrium is determined. The lower hygroscopicity of the flour with an initial moisture content of 12.2% determined at 37°C (98.6°F) as compared with 25°C (77°F) would of course be anticipated from the fact that adsorption reactions are characterized by a negative temperature coefficient. The hygroscopicity

values for 25°C (77°F) are not in the order of the original moisture contents of the flour, the sample of 12.2% moisture having the highest and that of 14.7% moisture the lowest hygroscopicity. Whether or not this displacement is due to the conditioning of the high-moisture sample at 2°C (35.6°F) (in order to preclude mold development), whereas the sample at 6.5% was produced by air-drying at room temperature, it is impossible to say but the data indicate that, in common with other biocolloids, relatively minor changes in treatment have an important influence on the hydration capacity of flour. In these experiments, an S-shaped curve best expresses the relationship between relative humidity and hygroscopicity, whereas Bailey (1920) secured curves having the shape of a simple parabola and Fairbrother (1929) found a linear relationship over a similar range in relative humidity. In a theoretical discussion of the water relationships in colloids, Briggs (1931) points out from thermodynamical and other considerations that S-shaped curves are predicated. In the instance of elastic gels at low vapor pressure the adsorption of water by the colloid is of greater importance than the influence of ionic constituents present in the gel; with increasing vapor pressure the adsorptive effect of the colloid becomes relatively insignificant as compared with that due to the effect of the ionic constituents in lowering the vapor pressure. The change in a biocolloid is thus the sum of the water-binding capacity of the colloid itself and of the ions which are present in the mass. In consequence, S-shaped curves result and curves of this type have been reported in the literature for a large number of biocolloids.

Discussion

This study, conducted under carefully controlled conditions, shows that flour responds rapidly to changes in relative humidity and that the rate of loss in moisture greatly exceeds the rate of regain. Substantiation in this regard is afforded by similar observations of Fairbrother (1929), who suggested that even at normal temperatures the drying of flour apparently permanently reduces its hydration capacity. Not only does the apparent hygroscopicity decrease with increasing temperature at which the hydration capacity is determined but even at constant temperature the hygroscopicity of a particular flour at a particular relative humidity is not a definite quantity, but depends upon its past history. In view of our knowledge of the effect of age on the hydrophilic properties of biocolloids, it would furthermore be surprising if a given wheat flour stored at a definite moisture and temperature showed constant hygroscopic equilibria values over any extended period of time. The studies reported in this paper were conducted with only one flour and there is reason to believe that flours of varying chemical composition

under precisely similar conditions of storage would yield different hygroscopic values. The data of Coleman and Fellows (1925), Fairbrother (1929), and Pap (1931) reveal that different wheats show considerable variation in their hygroscopicity and on storage of different wheats of varying moisture content together, equality in moisture content is not attained. In view of these considerations and the differences in experi-

TABLE VII
HYGROSCOPICITY OF WHEAT FLOUR AS REPORTED BY DIFFERENT WORKERS

Relative humidity	Flour moisture content				
	Bailey (1920)	Fairbrother (1929)	Anderson (1937)	Anker, Geddes and Bailey	
				Highest values ¹	Lowest values ²
%	%	%	%	%	%
30	5.2	9.4	—	9.7	8.5
40	6.5	9.7	—	11.1	9.9
50	8.0	10.7	12.0	12.3	11.1
60	9.8	12.8	13.7	13.2	12.3
70	12.1	14.0	15.4	14.5	13.7
80	15.0	15.7	—	16.3	15.8

¹ Tests at 25°C (77°F) with flour containing 12.2% moisture.

² Tests at 37°C (98.6°F) with flour containing 12.2% moisture.

mental technique, it is not surprising that wide discrepancies exist in the hygroscopicity of flour as reported by different workers. The available data known to the authors for selected humidities are summarized in Table VII. Approximate hygroscopic equilibria may also be computed from the storage tests; the best bases for this purpose would appear to be the mean values for the 5- and 10-pound paper sacks for those storage periods where the changes in weight are reasonably stable. These are compiled in Table VIII and compared with the equilibrium

TABLE VIII
COMPARISON OF HYGROSCOPIC EQUILIBRIA FROM STORAGE TESTS ON 5- AND 10-POUND PAPER AND COTTON SACKS AND LABORATORY SCALE STUDIES

Relative humidity	Approximate equilibrium moisture			Storage periods included in computation of second column
	Storage tests at 21.1-21.7°C	Laboratory tests at 25°C, flour at		
		12.2% moisture	14.7% moisture	
%	%	%	%	
36	9.8	10.5	9.7	5 to 10 weeks
45	11.3	11.7	10.9	7 to 9½ weeks
59	13.2	13.1	12.8	5 to 8 weeks
72	14.4	14.7	14.5	5 to 9½ weeks

values determined at 25°C (77°F) for the flours at 12.2% and 14.7% moisture as read from Figure 2. In view of the differences in temperature and original moisture content of the flour in the storage trials and laboratory tests, the agreement is perhaps all that could be expected, with the exception of the values for 36% relative humidity. It will be recalled that the flour used in the storage trials at this humidity level was first exposed to 30% and 65% relative humidity.

The observations made in these studies are of considerable practical significance to millers. Data collected from a large number of mills located in various sections of the United States concerning the moisture content of flour at the time of packing are now being studied in detail

TABLE IX

PERCENTAGE OVERPACKING REQUIRED TO INSURE 100% OF THE REQUIRED WEIGHT
AT VARYING RELATIVE HUMIDITIES WITH FLOUR PACKED AT
MOISTURE CONTENTS OF 13.0% TO 15.0%

Relative humidity	Flour moisture at equilibrium	Percentage overpacking required to provide full net weight for flours packed at following moisture contents.				
		13.0%	13.5%	14.0%	14.5%	15.0%
%	%	%	%	%	%	%
10	5.9	8.2	8.8	9.4	10.2	10.7
20	8.1	5.6	6.2	6.9	7.6	8.1
30	9.6	3.9	4.5	5.1	5.8	6.4
40	11.1	2.2	2.8	3.4	4.0	4.6
50	12.2	0.9	1.5	2.1	2.7	3.3
60	13.2	-0.2	0.3	0.9	1.5	2.1
70	14.5	-1.7	-1.2	-0.6	0.0	0.6
80	16.5	-4.0	-3.5	-2.9	-2.4	-1.8

to determine the regional and seasonal variations in the moisture content of different types of flour. A survey of the data shows that flours manufactured for the family trade range in moisture content, as milled, from approximately 13.0% to 14.0% moisture, the average being probably closer to the higher value. These flours are normally packed in small-sized paper and cotton sacks and hence are extremely susceptible to changes in weight due to atmospheric conditions. If we assume an average moisture content of 13.5% for the flour as packed, our hygroscopicity data indicate that the flour would have to be maintained at a relative humidity of at least 60% to prevent loss in weight. In many districts of the United States the humidity in stores and heated warehouses may fall much below this value for considerable periods, especially in the colder parts of the country during the winter months. The results of our studies and those of Fairbrother (1929) show that moisture will be lost rather rapidly but will be slowly and incompletely re-

gained upon re-exposure to higher humidities, thus likely resulting in a permanent weight loss.

According to the Federal Standards, flour may be packed at moistures up to 15.0% as determined by the vacuum or 130°C air-oven methods. It is therefore of interest to calculate the extent of overpacking flour which would be necessary to ensure 100% of the required weight at varying relative humidities for flours packed at moisture contents of 13.0% to 15.0%. For the purpose of these calculations, which are summarized in Table IX, the highest hygroscopic equilibria values obtained in our studies have been employed, namely those obtained at 25°C with flour at 12.2% initial moisture. These figures are only illustrative. Lower hygroscopicity values would result from exposure at higher temperatures and probably also with increasing age of the flour. In view of the slow rate of regain and the probable permanent reduction in hydration capacity it would be essential, in order to ensure full net weight, to overpack sufficiently to provide for the lowest relative humidity and the highest temperature at which the flour is ever likely to be exposed. This would imply that a large proportion of the flour packed for the family trade would reach the consumer overweight.

Summary

Studies have been conducted of the changes in net weight and moisture content of an 83% patent flour (commercially milled for the family trade from a blend of hard red spring and hard red winter wheats) and containing 13.3% to 13.4% moisture as packed in 5-, 10-, and 24½-pound paper and cotton bags and stored in air-conditioned cabinets maintained at 70°–71°F (21.1°–21.7°C) and relative humidities of 36, 45, 59, and 72% respectively with an accuracy of approximately $\pm 1^\circ\text{F}$ and 2% to 3% relative humidity.

Rate of change in weight was relatively rapid during the first several days of storage, the actual rate for any one type of container depending upon the magnitude of the difference between the original moisture content of the flour and the moisture content which would finally be attained at the existing relative humidity.

Initial response in weight to any storage condition depended upon the size and kind of package, the rate of change decreasing with the package size and being greater for cotton than for paper sacks. As the storage period was prolonged the cumulative percentage changes in weight for the various containers tended to equalize and approach a common value. No definite equilibrium was obtained even after eight to ten weeks' storage.

Rate of moisture loss on exposure to low relative humidity is much more rapid than the rate of regain; the partial drying of flours even at atmospheric temperatures apparently permanently reduces their hydration capacity.

The approximate moisture equilibria obtained in the storage trials at 70°–71°F (21.1°–21.7°C) for 36, 45, 59, and 72% relative humidity were 9.8, 11.3, 13.2, and 14.4% moisture respectively as determined by the 130°C air-oven method.

Laboratory studies of the hygroscopic equilibria of the same flour brought to initial moistures of 6.5, 12.2, and 14.7% conducted at 25°C (77°F) over the range of 10% to 80% relative humidity revealed that the equilibrium is influenced either by the initial moisture content of the flour or by the environmental conditions to which the flours were exposed during conditioning. Flour at 6.5% moisture had the lowest hygroscopicity and at 12.2% moisture the highest, the differences at corresponding humidities being approximately 1.0%.

Hygroscopic equilibrium of flour at constant relative humidity varies with temperature. Comparative tests at 25°C (77°F) and 37°C (98.6°F) gave differences up to approximately 1.0% at certain humidities.

An S-shaped curve best expressed the relation between relative humidity and hygroscopicity, and is in accord with theoretical considerations of the water relationships of colloids.

A given flour does not possess a definite hygroscopicity, the hydrophilic properties being influenced by its past history.

The practical implications of these studies in relation to the changes in weight of packaged flour are discussed.

Acknowledgment

The authors gratefully acknowledge the cooperation of the Minnesota Department of Weights and Measures in making their official Gurley precision balance available for this research and for adjusting and calibrating the weights used.

Literature Cited

- Anderson, J. E.
1937 Equilibrium relative humidities for hard wheat and mill stocks. *Northwestern Miller* 192: 20–22.
- Arpin, M., and Pécaud, M. T.
1923 Variations dans le poids des farines. *Ann. fals.* 16: 586–597.
- Bailey, C. H.
1920 The hygroscopic moisture of flour exposed to atmospheres of different relative humidity. *Ind. Eng. Chem.* 12: 1102–1103.
- Briggs, D. R.
1931 Water relationships in colloids. I. Vapor pressure measurements on elastic gels. *J. Phys. Chem.* 35: 2914–2929.
- Cathcart, W. H., and Killen, E. J.
1939 Changes in flour on storage with special reference to the effect of different types of bags. *Cereal Chem.* 16: 798–817.

- Coleman, D. A., and Fellows, H. C.
1925 Hygroscopic moisture of cereal grains and flaxseed exposed to atmospheres of different relative humidity. *Cereal Chem.* 2: 275-287.
- Fairbrother, T. H.
1929 The influence of environment on the moisture content of flour and wheat. *Cereal Chem.* 6: 379-395.
- Fisher, E. A.
1927 A study of the rate of drying of wheat flour, starch, and gluten. *Cereal Chem.* 4: 184-206.
- Frank, W. L.
1923 Short weights of flour and feed. *J. Am. Assn. Cereal Chemists* 8: 19-47.
- and Campbell, R. L.
1922 Moisture content vs. net weight. *National Miller* 27: 40-41.
- Guthrie, F. B., and Norris, G. W.
1912 Daily variation in moisture content of flour. Wheat and flour investigations. Dept. Agr. New South Wales Science Bul. 7: 18-20.
- Herman, R. S., and Hall, W.
1921 Variation in moisture content of flour during storage. *J. Am. Assn. Cereal Chemists* 6 (No. 3): 10.
- Krtinsky, K.
1937 Mehllagerung in Jute- und Papiersäcken. *Mühlenlab.* 7: 131-134.
- Pap, L.
1931 Hygroscopicity of wheat. *Cereal Chem.* 8: 200-206.
- Sanderson, T.
1914 A study of the variation in weight of a fifty-pound sack of flour during storage. *N. Dak. Agr. Exp. Sta. Special Bul.* 3: 14-31.
1915 A further study of the variation in weight of a fifty pound sack of flour during storage. *N. Dak. Agr. Exp. Sta. Special Bul.* 3: 250-259.
- Smith, E. R., and Mitchell, L. C.
1925 Observations on the drying of flour (with notes on its hygroscopic properties). *Ind. Eng. Chem.* 17: 180-183.
- Stockham, W. L.
1917 The capacity of wheat and mill products for moisture. *N. Dak. Agr. Exp. Sta. Bul.* 120.
- Swanson, C. O., Willard, J. T., and Fitz, L. A.
1915 Kansas flours. Chemical, baking and storage tests. *Kan. Agr. Exp. Sta. Bul.* 202: 119-135.
- Thiessen, E. J.
1933 Effect of storage upon the bread-making qualities of Wyoming hard wheat flour. *Wyo. Agr. Exp. Sta. Bul.* 198: 3-27.
- Willard, J. T.
1911 Changes in the weight of stored flour and butter. *Bul. Kansas Board of Health* 7: 9-14.
- Wilson, R. E.
1921 Humidity control by means of sulphuric acid solutions, with critical compilation of vapor pressure data. *Ind. Eng. Chem.* 13: 326-331.

THE EVALUATION OF FLUOROPHOTOMETERS TO BE USED IN THE THIOCHROME ASSAY FOR VITAMIN B₁¹

JOHN R. LOOFBOUROW and ROBERT S. HARRIS

Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Massachusetts

(Read at the Annual Meeting, May 1941)

The present investigation was undertaken for the purpose of establishing the reliability and precision that may be expected in the thiochrome assay of vitamin B₁ with available commercial fluorophotometers as far as instrument performance is concerned. Five fluorophotometers of varying design and construction were tested for: (1) time required to attain stable operating conditions, (2) linearity of response with quinine sulfate, thiochrome, and thiochrome in flour eluate, (3) reproducibility of readings, (4) sensitivity, (5) effect of line voltage fluctuations, (6) fatigue, and (7) rate of destruction of thiochrome.

Methods of Exciting and Measuring Fluorescence in Fluorophotometers

The general methods of investigating fluorescence in biochemical substances have been reviewed in detail by Dhéré (1937). In order to excite fluorescence, the substance, generally in solution, must be illuminated with light of appropriate wave length. For the fluorophotometric assay of thiochrome, the mercury arc lines in the ultraviolet at 365 m μ furnish satisfactory exciting radiation. High-pressure mercury arcs of the H-3 or H-4 type have strong lines in this region of the spectrum, and are customarily employed. It is not necessary that they be in quartz envelopes, as glass is sufficiently transparent at 365 m μ . Mercury arcs with quartz envelopes (*e.g.*, those of the "Uviarc" type) may, however, be used.

The radiation from all of these sources contains much energy in the visible part of the spectrum as well as in the ultraviolet, and it is necessary to eliminate the visible rays as efficiently as possible in order to prevent their effect from being added to that of the fluorescent visible light. For this purpose, a "primary filter," which transmits ultraviolet but absorbs visible radiation, is used. Several filters are available for this purpose. The four used in the instruments investigated are Corning Nos. 584, 586, 587, and 597, of which the transmission curves are shown in Figure 1. In general, those which transmit more of the ultraviolet also transmit more radiation at the

¹Contribution No 192 from the Department of Biology and Public Health, Massachusetts Institute of Technology.

blue end of the visible spectrum—hence increase in exciting energy is attained only with sacrifice of spectral purity.

The photocell for measuring the intensity of the fluorescent light is customarily placed at the side of the cuvette containing the fluorescing solution so as to minimize the amount of radiation falling on it from the primary ultraviolet beam. Nevertheless, the primary energy scattered into the photocell is of sufficient magnitude so that its effect must be reduced, as far as practicable, by "secondary filters" which transmit the fluorescent light freely but absorb the primary ultraviolet.

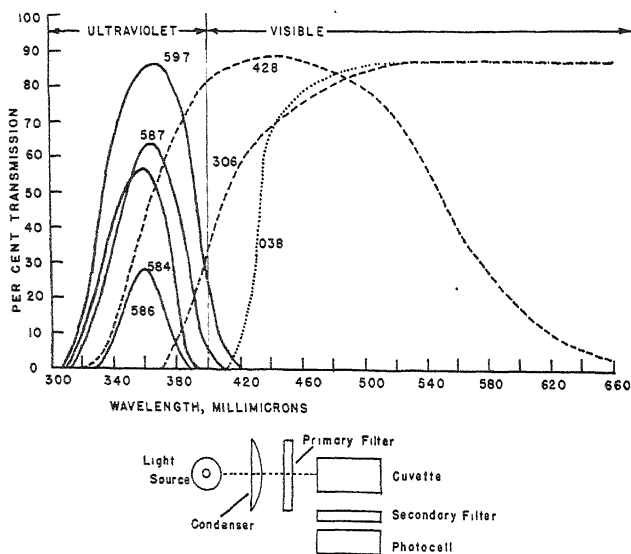


Fig. 1 Set-up for photoelectric fluorophotometry, and transmission curves of filters used in the thiochrome assay of vitamin B₁.

One of two alternative secondary filter systems is ordinarily employed. The first consists of a Corning Type 038 followed by a Type 428. The 038 absorbs efficiently all ultraviolet of wave length shorter than about 410 mμ, but the absorbed ultraviolet causes this filter to emit yellow fluorescent light. The Type 428 filter absorbs part of the fluorescent radiation from the 038 filter, but transmits the fluorescent radiation from thiochrome. The combination effectively eliminates most of the scattered ultraviolet from the primary beam. If, instead of this combination, a Type 306 or 038 Corning filter is used alone, somewhat greater transmission of the fluorescent light from the

solution is obtained, but this is diluted by the entire fluorescent light from the secondary filter. The absorption curves of the secondary filters customarily used are shown in Figure 1, and the primary and secondary filter combinations recommended by the manufacturers for the various instruments investigated are shown in Table I.

TABLE I
INSTRUMENT MANUFACTURERS' RECOMMENDED FILTER COMBINATIONS

Instrument	Light source	Primary filter	Secondary filters (in order from cuvette to photocell)
A	H3	Corning 584	Corning 038, 428
B	"Uviarc"	Corning 586	Corning 038, 428
C	H4	Corning 597	Corning 306
D	H4	Corning 587	Corning 038, 428
E	H4	Corning 587	Corning 038, 428

Photocells and Electrical Circuits Used in the Fluorophotometers Investigated

Two types of photocells are employed in existing fluorophotometers: barrier layer and photoemissive cells. The former have the advantages of being self generating (that is, of needing no external battery or source of emf) and of having high sensitivity. They are, however, subject to fatigue, especially if exposed to considerable infrared radiation, and they give linear response in relation to light intensity only when used with low resistance loads and at low light levels. Photoemissive cells have comparatively linear response with high resistance loads and are free from fatigue, but they must be used in connection with accessory electronic amplifiers. Cells of either type may be employed satisfactorily for fluorescence measurements. When barrier layer cells are used, the investigator should be aware of their liability to fatigue. Because of the low light levels characteristic of fluorescence, however, fatigue is only of consequence in fluorescence measurements when instruments are used continuously for extended periods of time.

In the simplest circuits, a barrier layer photocell is directly connected to a sensitive galvanometer, and the galvanometer deflection is calibrated in terms of solution concentration. A variable shunt is provided for adjusting the sensitivity of the galvanometer, and a battery and potentiometer may be employed for surpressing the zero, so that the readings of the blank are zero. In order to obtain greatest linearity of response, the galvanometer should have a resistance of less than 1,000 ohms (Lange, 1938). The galvanometer sensitivity required can be calculated from our measurements, which indicate

that with thiochrome equivalent to a total of 0.1 μg of thiamin in the cuvettes of the various fluorophotometers investigated, the light intensity on the photocells is of the order of 2×10^{-5} lumens per square centimeter of photocell surface. The barrier layer cells usually employed have sensitivities of the order of 500 microamperes per lumen and surface areas of about 10 square centimeters. For readings of 20-scale divisions for 0.1 μg of thiamin it is therefore necessary to use galvanometers having sensitivities of the order of $2 \times 10^{-5} \times 10 \times 500/20 = 5 \times 10^{-3}$ microamperes per scale division.

TABLE II
PHOTOCELLS AND CIRCUITS EMPLOYED WITH FLUOROPHOTOMETERS TESTED

Instrument	Photocells	Type of circuit
A	1 barrier layer	Cell directly connected to sensitive, low-resistance galvanometer.
B	3 barrier layer (2 for fluorescent pickup, 1 for balancing)	Current balancing null circuit (Brice).
C	2 barrier layer	Potentiometric null circuit.
D	1 barrier layer	Potentiometric null circuit using battery for potentiometric voltage.
E	1 photoemissive (RCA 929)	Single stage electronic amplifier and output meter.

Barrier layer photocells may also be used in circuits in which the current or potential developed by the photocell is balanced against an opposing current or potential from a battery or from a comparison photocell placed in a part of the light beam. Various circuits have been devised for this purpose, some of which are especially designed to cause the photocells to operate into low resistances at balance, so that their output will be approximately a linear function of light intensity. Such circuits have been reviewed by Müller (1939). One advantage of using two photocells in a null circuit is that variations in readings due to fluctuations in line voltage (which are, of course, reflected as fluctuations in intensity of the light source, and therefore as changes in fluorescent light) may be eliminated almost completely in this way.

When photoemissive cells are used, the current from them may be amplified by a single-stage electronic amplifier and measured in terms of amplifier plate-circuit current.

It is not the purpose of the present paper to give details of circuit design. The photocells and general types of circuits used in the instruments investigated are summarized in Table II.

Time Required to Attain Stability of Operation

The mercury-vapor lamps used as ultraviolet sources in fluorophotometers require considerable time to attain their full operating temperature. During this preliminary period, the intensity of their emitted radiation constantly increases. It is important, therefore, to permit full operating intensity to be attained before readings are taken. The following experiments were conducted to determine how long an initial heating period is required before the maximum intensity level is reached.

Using two instruments (A and E) which did not employ balancing photocells, a solution of quinine sulfate solution (concentration $0.27 \mu\text{g}$ per ml) was added to the cuvettes,² and the instruments were adjusted to give full-scale deflection after a stabilizing period of at least half an hour. The lamps were turned off and allowed to cool to room temperature. They were then turned on again and readings were taken at intervals of one minute from the moment of lighting until stability had been attained.

The results for instrument A, incorporating an H-3 type mercury arc, and for instrument E, employing an H-4 type mercury arc, are shown in Figure 2. It is evident that in both instances full operating intensity was reached before ten minutes had elapsed. In the case of the H-4 lamp, the intensity rose somewhat more quickly, reaching its maximum in six minutes. These curves should not be taken as quantitative comparisons of the characteristics of H-3 and H-4 lamps, however, as the rate of temperature rise in each instance depends somewhat upon the type of lamp housing and ventilation employed. In the cases illustrated, the H-4 lamp was cooled by a fan, whereas the H-3 was not.

In order to see whether the rise of intensity would be more rapid if the lamps were not cooled completely to room temperature before relighting, the H-4 current supply was turned off at ten minutes and immediately turned on again. It is a characteristic of lamps of this type that they do not relight immediately when hot. It was found that the H-4 lamp would relight after a cooling period of about two

² The greater photochemical stability of quinine sulfate solutions than of thiochrome solutions makes it advantageous to use the former as the fluorescing material in instrument tests. It seemed desirable, however, to use quinine sulfate at concentrations easily translatable into thiamin equivalents. The quinine sulfate secondary standards recommended by the various instrument manufacturers ranged in concentration from 0.20 to $0.27 \mu\text{g}$ per ml. It was found in our tests that the higher limits of these concentrations gave a fluorescent response with the particular instruments tested approximately equivalent to that of $1 \mu\text{g}$ of thiamin oxidized and taken up as thiochrome in 10 ml of butanol, that is, of $0.1 \mu\text{g}$ of thiamin per ml of final thiochrome solution. It should be noted that, provided a minimum volume of thiochrome solution is used sufficient to fill the cuvettes to the proper height, the response of fluorophotometers is a function of the final thiochrome concentration only, and $1 \mu\text{g}$ of thiamin oxidized and taken up in 10 ml of butanol gives the same response as $1.5 \mu\text{g}$ oxidized and taken up in 15 ml . Since we found that a $0.27 \mu\text{g}$ quinine sulfate solution in $0.1N \text{ H}_2\text{SO}_4$ gave a fluorescent response equivalent to that of a thiochrome solution corresponding to $0.1 \mu\text{g}$ of thiamin per ml of final thiochrome solution, this concentration of quinine sulfate was adopted as the secondary standard for use in our tests. The $0.27 \mu\text{g}$ per ml quinine sulfate solution was made up by 40-to-1 dilution of a stock solution of $10.8 \mu\text{g}$ per ml.

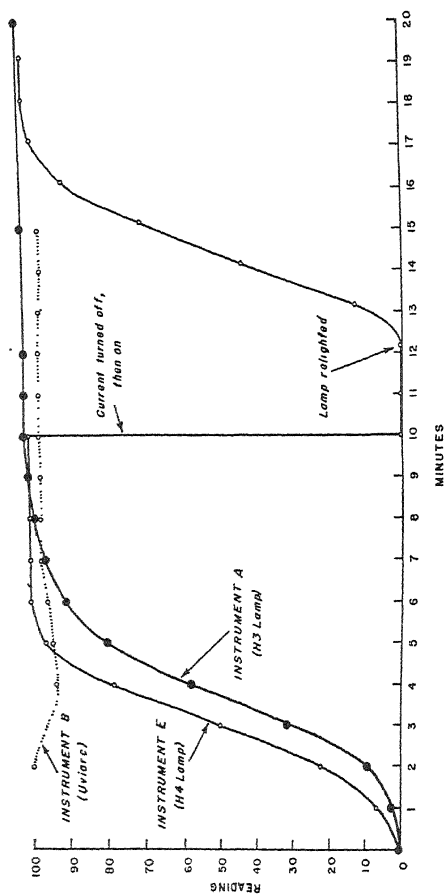


Fig. 2. Heating-time tests using quinine sulfate at 0.27 μg per ml in 0.1N H₂SO₄.

minutes in the particular lamp housing used. As shown by the figure, however, the rate of increase in intensity after relighting was almost identical with that found for the same lamp started at room temperature.

In the case of fluorophotometers in which line voltage fluctuations are balanced by a second photocell illuminated by a part of the radiation from the lamp, stability of operation might be expected as soon as the lamp had attained sufficient intensity for satisfactory null readings to be obtained with the potentiometer. Tests of the time required to attain stability for a fluorophotometer of this type (instrument B) are shown in Figure 2. It was not possible to balance the fluorophotometer until two minutes after the lamp had been turned on. The null reading then obtained was at 100 on the potentiometer scale, the value to which the instrument had been adjusted when the lamp was fully heated. During the next two minutes, there was a slight decrease in readings, and subsequently a slow rise until after seven minutes the readings remained constant. While the initial heating period makes much less difference in the readings of instruments of this type, it is evident that it is better, even with balanced circuits, to allow time for stable operating conditions to be attained.

It appears from these results that the initial "warming-up" periods of 30 minutes to one hour used by many workers is unnecessarily long. A heating period of 10 to 15 minutes seems entirely adequate for mercury arcs of the types usually employed in fluorophotometric investigations.

Linearity of Response, General Considerations

It is desirable that the readings obtained be directly proportional to the concentration of the fluorescing material. If a linear relationship obtains between readings and concentration, the calibration of the instrument is considerably simplified and the accuracy with which interpolation can be made between calibrated points is increased. If readings are a strictly linear function of concentration, one has but to obtain two values, r_1 and r_2 , for two known concentrations, c_1 and c_2 , following which the concentration X of any unknown solution may be determined from the corresponding net reading (minus the blank) R , by simple proportion.

$$X = R \frac{c_1 - c_2}{r_1 - r_2}.$$

Linearity of response may be achieved if (1) the intensity of fluorescent light is directly proportional to the concentration of fluorescing material, and (2) the response of the instrument is a linear function

of the light incident on its photocells. In order for the first condition to be met, it is necessary that the concentration of the solution be sufficiently low so that it absorbs inappreciably either exciting radiation or fluorescent light. For the second condition to be met, it is necessary that the electrical design of the instrument be such that the fluorescent light is converted into an electrical current or voltage directly proportional to it. This may be achieved in the case of cells of the photo-emissive type by the use of carefully designed amplifier circuits, and in the case of those of the barrier layer type by careful choice of cells and by using them only with low resistance loads and operating them at low light-intensity levels.

The limiting concentration above which there is appreciable departure from linearity of fluorescent light intensity as a function of concentration is also related to the shape and size of the cuvette. The longer the path of incident and fluorescent light through the cuvette, the greater the absorption of light will be. Doubling the path length d is equivalent to doubling the concentration c insofar as influence on absorption is concerned, as is evident from the following expression (the Lambert-Beers absorption law) for the absorption of light of a given wave length:

$$I_d = I_0 e^{-kcd},$$

where I_0 and I_d are respectively the light intensity before and after traversing a path length d through the solution, e is the base of Napierian logarithms, c is the concentration, and k is the absorption coefficient of the solution at the particular wave length in question, in units corresponding to those employed for c and d . It would appear, therefore, that to increase the range of linearity, the smallest possible cuvettes should be used. On the other hand, reduction in cuvette size, with the concentration held constant, results in decrease in the light available for operating the photocells. There are, therefore, practical limits beyond which this method of remedying the situation cannot be carried.

Linearity of Response with Quinine Sulfate

The linearity of response was tested with two series of dilutions of quinine sulfate in 0.1N H₂SO₄, one ranging in concentration from 0.027 to 0.27 μg per milliliter (corresponding to thiamin hydrochloride equivalent to 0.01 to 0.1 μg per ml of thiochrome solution) and the other ranging from 1.08 to 10.8 μg per ml (corresponding to thiamin hydrochloride equivalent to 0.4 to 4.0 μg per ml of thiochrome solution). In both instances, the sensitivities of the instruments were adjusted, when possible, to give approximately full-scale reading for

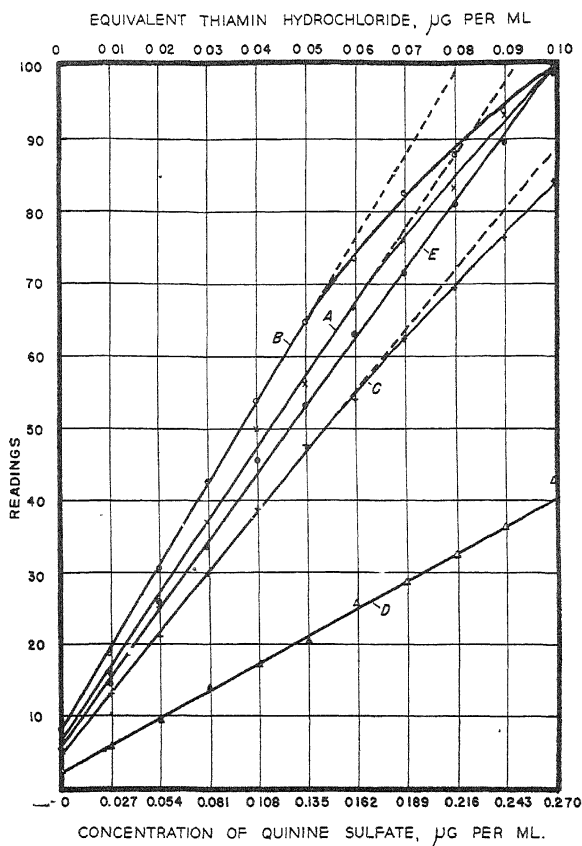


Fig. 3. Tests of linearity with quinine sulfate, 0 to 0.27 μg per milliliter.

the maximum concentration employed. The results for the lower concentration range are shown in Figure 3 and those for the higher range in Figure 4. All readings are given on the basis of 100 for full scale. All of the instruments tested gave approximately linear response up to a level of 0.162 μg of quinine sulfate per ml (corresponding to 0.06 μg of thiamin hydrochloride per ml of thiochrome solution) and two of them (D and E) gave linear response to considerably higher levels. In the higher concentration range, the departure from linearity was considerably more marked (Fig. 4), but since these levels corresponded to greater concentrations of thiochrome than one would

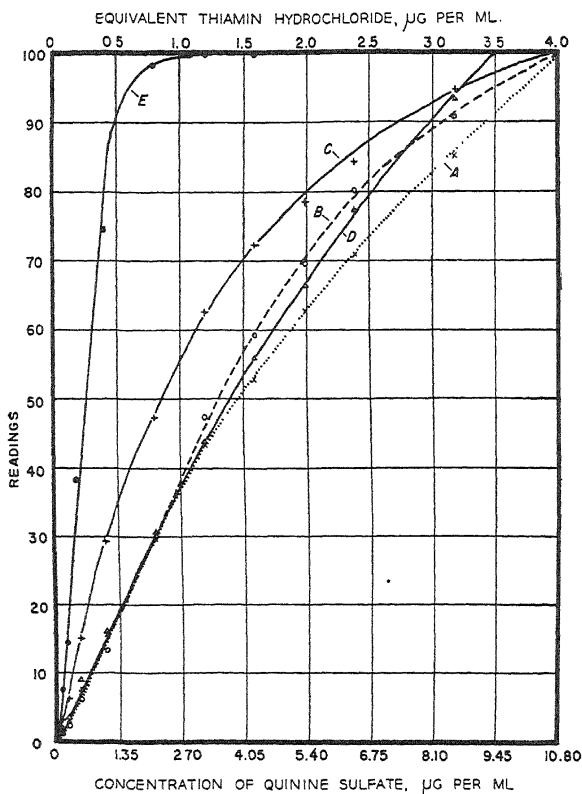


Fig. 4. Tests of linearity with quinine sulfate, 0 to 10.8 μg per milliliter.

usually wish to test, the lack of linearity under these conditions is not particularly important.

With one instrument (C), which employs a quinine sulfate comparison standard to excite the balancing photocell, it was found necessary to remove this standard between readings in order to avoid errors due to photochemical deterioration of the standard.

Linearity of Response to Graded Amounts of Thiochrome

To test the linearity of response with thiochrome, a thiochrome solution was prepared as follows:

One milliliter of a stock solution of thiamin prepared in accordance with USP XI procedure (2nd supplement, pp 131-2) and containing 500 μg per ml was diluted to 100 ml with 0.1N sulfuric acid. Four ml

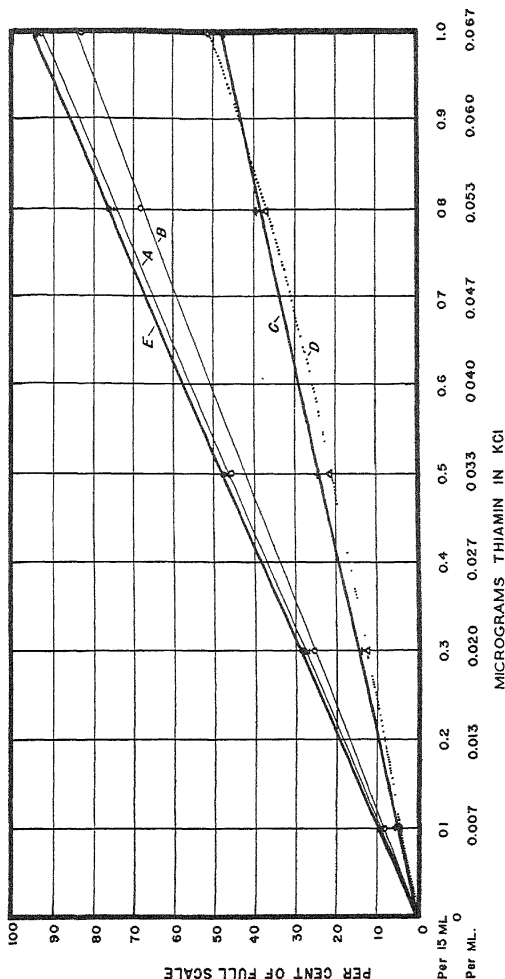


Fig. 5. Tests of linearity with thiochrome in butanol.

of this dilution was diluted to 100 ml with 25% potassium chloride solution in 0.1*N* hydrochloric acid. 5.0-ml aliquots from this KCl dilution (each aliquot containing a total of 1.0 μ g of thiamin) were put into two separatory centrifuge tubes. To the first, 3 ml of a 15% sodium hydroxide solution containing 0.03% potassium ferricyanide were added and quickly mixed. To the second, 3 ml of 15% sodium hydroxide were added in the same way. Fifteen ml of distilled iso-

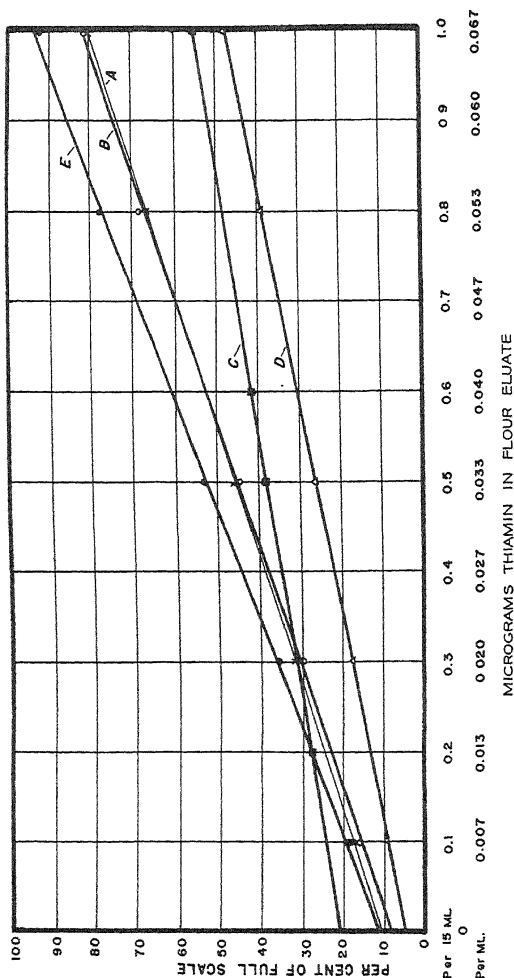


Fig. 6. Tests of linearity with thiochrome in flour eluate.

butanol were added to each, the tubes stoppered, shaken vigorously for $1\frac{1}{2}$ minutes, then centrifuged at 500–600 rpm for $\frac{3}{4}$ minute in upright position. The stoppers were removed and the lower aqueous layer discarded. Two grams of anhydrous sodium sulfate was then added to each tube, the tubes shaken vigorously for a moment, and the contents poured into containers appropriate for reading of the instruments.

A series of solutions prepared from this dilution of the stock thiamin solution and containing graded levels of thiamin was oxidized, then tested on each instrument (Fig. 5). The response was approximately a linear function of concentration in each case.

Linearity of Response to Graded Amounts of Oxidized Thiamin Dissolved in Flour Eluate

The experiments with thiochrome were repeated using a composite extract of flour eluted from exchange zeolite with 25% potassium chloride. These eluates were prepared from four samples of flour in accordance with the procedure outlined by Hennessy (1941) with the exception that the flour solution contained 1.7% rather than 1.22% of sodium acetate.

The readings obtained with graded amounts of thiochrome in flour eluate are shown in Figure 6. All instruments responded in direct proportion to concentration.

The data of Figures 4, 5, and 6 indicate that all five instruments can reliably measure thiochrome in a cuvette or tube in concentrations corresponding to between 0.1 and 1.0 μg of thiamin hydrochloride per 15 ml of thiochrome solution. They indicate, further, that all instruments can rather accurately measure as little as 0.1 μg of thiamin per 15 ml of thiochrome solution in pure solution or in the presence of flour eluate.

Reproducibility of Readings

The reproducibility of readings was tested by making repeated determinations on a series of dilutions of quinine sulfate in 0.1*N* sulfuric acid ranging in concentration from 0.027 to 0.27 μg per milliliter. The instruments were adjusted, so far as possible, to give approximately full-scale reading with the dilution of maximum concentration. The line voltage was kept constant at 110 ± 0.25 volts.

The purpose of these experiments was to determine the magnitude of deviations (such as those due to zero drift of meters, variations in the setting of potentiometers at the null point, errors in estimating fractions of a scale division, etc.) which have a magnitude independent of the intensity being read. These may be expressed as percentages of full-scale deflection, or as fractions of a scale division. For the data shown in Table III, the deviations from the means were calculated for 20 readings. The table shows for each instrument the maximum average, and root mean square (standard) deviations. The root mean square deviation, σ , where

$$\sigma = \sqrt{\frac{\sum(M - x)^2}{n}}$$

M is the mean of readings, x is the value of each individual reading, and n is the total number of readings (in this case 20), is useful because, if we assume that the frequency of errors of various magnitudes follows the "normal curve of error," one can calculate from the standard deviation, σ , the probability of any reading deviating by more than a particular amount from the mean due to inherent errors of the instrument. Thus there is a 50% chance of readings deviating more than 0.675σ from the mean. This value is known as the probable error, P , and is shown in the last column of Table III.

TABLE III
PROBABLE ERRORS OF READINGS IN FRACTIONS OF A SCALE DIVISION¹
(On basis of full scale = 100 divisions)

Instrument	Deviation from mean (20 values)			Probable error of readings, P^1
	Maximum	Average	Standard	
A	± 1.0	± 0.63	± 0.68	± 0.46
B	1.9	1.06	1.17	0.79
C	1.7	1.00	1.04	0.70
D	2.3	0.95	1.09	0.74
E	1.0	0.44	0.51	0.34

¹ The probable error is 0.675 times the standard deviation, and is the limit of deviation within which 50% of random readings may be expected to lie.

Sensitivity

All instruments were adjusted to give as large a reading as possible with quinine sulfate at $0.027 \mu\text{g}$ per ml (equivalent to $0.01 \mu\text{g}$ thiamin hydrochloride per ml of thiochrome solution). From the readings R so obtained, the readings of the corresponding blanks B (cuvette filled with $0.1N \text{H}_2\text{SO}_4$) were subtracted. These results were used to calculate the sensitivity S in μg per ml per scale division (Table IV).

TABLE IV
TESTS OF SENSITIVITY WITH QUININE SULFATE

Instrument	Micrograms per milliliter per scale division at maximum sensitivity ¹	
	Quinine	Thamin equivalent ²
A	0.0014	0.00051
B	0.0013	0.00047
C	0.00092	0.00034
D	0.0078	0.0029
E	0.00078	0.00029

¹ Using the filters recommended by the manufacturers for thiochrome determinations (see Table II).

² Equivalent thiamin hydrochloride per ml of thiochrome solution.

$$S = \frac{0.027}{R - B}$$

By multiplying the probable error in scale divisions by the sensitivity per scale division one obtains the probable error in reading the material in question at maximum instrument sensitivity. Such values for deviations of one to four times the probable error (corresponding to chances of 50% to 0.7%) are shown in Table V.

TABLE V
PROBABLE INSTRUMENT ERRORS IN TERMS OF THIAMIN

Instrument	Error in μg of thiamin per ml of thiochrome solution ¹ for various multiples of probable error P			
	P	$2P$	$3P$	$4P$
A	± 0.00023	± 0.00046	± 0.00069	± 0.00092
B	0.00037	0.00074	0.00111	0.00148
C	0.00024	0.00048	0.00072	0.00096
D	0.0021	0.0042	0.0063	0.0084
E	0.00010	0.00020	0.00030	0.00040
Probable chance of occurrence of error of magnitude shown				
	50%	17.7%	4.3%	0.7%

¹ Equivalents of the quinine sulfate used in the determinations.

Effect of Line Voltage Fluctuations

In order to investigate the influence of line voltage fluctuations on the readings obtained with the various instruments, all instruments were adjusted to full scale deflection with the quinine sulfate at a concentration of $0.27 \mu\text{g}$ per ml in their cuvettes. Solutions of half this concentration were then substituted, and readings were taken at line voltages from 100 to 120. The voltage was regulated by means of an autotransformer (General Radio "Variac").

In all instances, the change in reading obtained was approximately a linear function of the change in line voltage (Fig. 7). In the case of instruments not employing balancing photocells to compensate for line voltage fluctuations, voltage changes caused variation in readings of the order of 2% to 4% per volt. In the case of instruments having balancing photocells, variations of the order of 0.1% to 0.15% per volt were obtained.

Values were also obtained after compensating line voltage fluctuation in the unbalanced instruments with a line voltage regulating transformer (Raytheon). Under these conditions fluctuations were reduced to about the same order as those experienced with balanced-circuit instruments. It appears that this method of line voltage control is satisfactory, if the frequency regulation of the supply line

is good. It is well known that changes in the frequency of the supply current lead to changes in the output of voltage regulating transformers. Consequently, the transformer compensation method should be used only in instances in which the line frequency regulation is adequate.

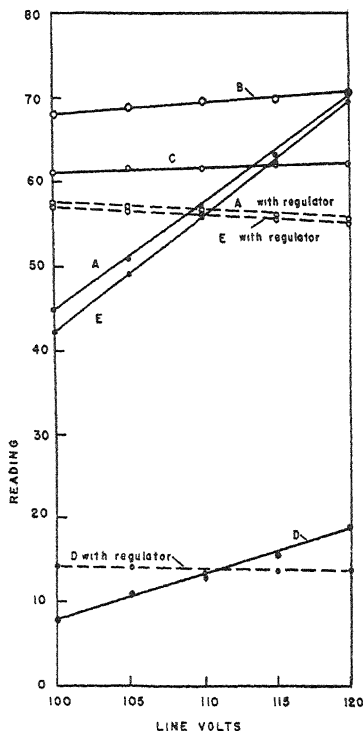


Fig. 7. Effect of line voltage variation on readings.

Relative Destruction of Thiochrome

The instruments were adjusted so far as possible to give full-scale readings with thiochrome in a concentration equivalent to 1.0 μg of thiamin hydrochloride per 15 ml of thiochrome solution in butanol. A fresh solution was then placed in each instrument, a reading was made as soon as possible, and subsequently $\frac{1}{2}$, 1, 2, 3, 5, and 8 minutes after insertion (Fig. 8).

In this experiment, instrument E was used as recommended; the button controlling the shutter was pushed down and the solution

exposed to radiation only when a reading was taken. The advantage of this procedure is shown by the results of this experiment. Similarly, the shutter in instrument A was opened only to take readings. The effect of leaving this shutter open continuously is shown by the lower curve for A, which indicates considerable thiochrome destruction. The small amount of destruction in instrument B is evidently the result of its use of a 586 type primary filter in combination with two photocells, which permits sufficient sensitivity to be obtained at low levels of illumination of the fluorescing solution.

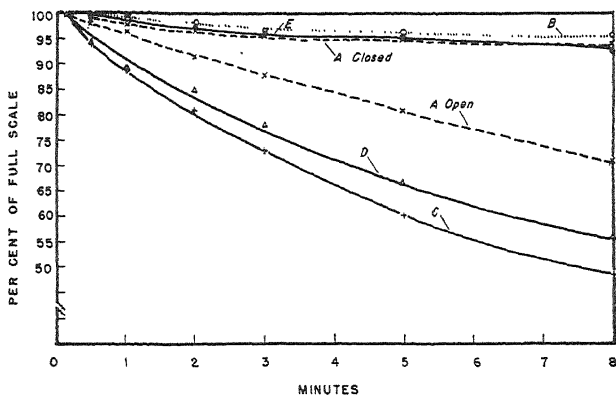


Fig. 8. Comparative rates of destruction of thiochrome.

In both instruments D and C the thiochrome is exposed to considerable radiant energy. As there is no means of protecting the test solutions while these instruments are in use, considerable thiochrome destruction normally occurs. While it is a good routine practice to standardize the procedure and make readings at a definite interval after placing the solution in an instrument in any case, this precaution seems imperative when using instruments C and D.

It was not possible to make a true zero reading since about 5 seconds (E), 10 seconds (A, B, D), and 15 to 20 seconds (C) elapsed with the various instruments before a proper reading could be made. However, if one is allowed to project the curves in Figure 8 back to a zero observation, it appears that perhaps 5% of the thiochrome is destroyed in instruments C and D before a reading can be made. This is not serious if an identical procedure is used in standardizing the instruments with pure thiamin. A very serious error can result if timing is not strictly observed.

Conclusions

The results of these investigations indicate that:

1. A heating period of 10 to 15 minutes is sufficient to attain stable operating conditions in the fluorophotometers tested.

2. All instruments tested have sufficient sensitivity, linearity, and reproducibility of readings to permit the evaluation of thiamin at levels of 0.1 to 1.5 μg per 15 ml of thiochrome solution with probable errors of the order of 0.0001 to 0.0021 μg of thiamin per ml of thiochrome solution.

3. The variation in readings due to line voltage fluctuations is of the order of 2% per volt at 110 volts in instruments without balanced photocell circuits. This is reduced to the order of 0.1% per volt in instruments employing balanced circuits, or in unbalanced instruments when used with line voltage regulating transformers on lines with good frequency regulation.

4. The rate of destruction of thiochrome in these instruments is insufficient to cause serious error in readings but in two instruments (C and D) it is high enough to make advisable the taking of readings at a constant time after introducing the sample into the instrument.

Acknowledgment

The assistance of the Research Corporation in supporting these investigations and the cooperation of the various equipment manufacturers in supplying instruments and accessory apparatus are gratefully acknowledged. Our thanks are due Mr. E. J. Josephson, Miss Lindy E. Marsh, Mr. Lloyd M. Mosher, Mr. C. V. Nelson, Mr. R. L. Sinsheimer, Mr. W. R. Slaunwhite, Jr., Mr. A. M. Webb, and Miss Elizabeth Weeks for their assistance during the course of the investigations.

Literature Cited

- Dhéré, Ch.
1937 *La fluorescence en biochimie*. Les Presses Universitaires de France, Paris.
- Hennessy, D. J.
1941 Chemical methods for the determination of vitamin B₁. *Ind. Eng. Chem. (Anal. Ed.)* 13: 216-218.
- Lange, B.
1938 *Photoelements and their applications*. Reinhold Publishing Company, New York.
- Müller, R. H.
1939 Photoelectric methods in analytical chemistry. *Ind. Eng. Chem. (Anal. Ed.)* 11: 1-17.

CEREAL CHEMISTRY

VOL. XIX

MARCH, 1942

No. 2

A COLLABORATIVE STUDY OF VARIOUS METHODS FOR ESTIMATING THE PIGMENT CONTENT OF FLOUR EXTRACTS¹

D. S. BINNINGTON

General Mills, Inc., Research Laboratories, Minneapolis, Minnesota

(Read at the Annual Meeting, May 1941)

In accordance with the recommendation of the 1939-40 Committee on Methods of Analysis, studies upon the determination of flour pigments have been extended to a collaborative investigation of various procedures for estimating the pigment content of flour extracts.

In this study, variables associated with preparation of the extracts themselves were eliminated by supplying the collaborators with aliquot portions of large samples of flour extracts prepared in one laboratory. In the majority of cases, naphtha + alcohol (93 : 7) was employed as solvent, since Ferrari and Croze² have shown that flour extracts prepared with this solvent are stable at room temperature for at least nine months if protected from excessive exposure to daylight. However, since several collaborators possessed instruments calibrated only for water-saturated butanol, extracts were prepared with this solvent also. As a check upon the stability of these extracts, samples were stored in clear glass bottles in a dark cupboard at room temperature for a period of 35 days. No change in pigment concentration could be detected.

The results obtained by eight collaborators using naphtha-alcohol extracts are given in Table I and the means for methods in Table II.

These results in general show good agreement, particularly when the wide variety of instruments and methods employed is taken into consideration. The spectrophotometric results show greater divergence than might be anticipated. Collaborators 5 and 6, however, obtained results in excellent agreement using full spectrophotometric

¹ Subcommittee Report, 1940-41 Committee on Methods of Analysis, Paper No. 26, Journal Series, General Mills, Inc., Research Laboratories.

² C. G. Ferrari and Alice B. Croze: A flour extract as a standard in the colorimetric estimation of flour color, *Cereal Chem.* 11: 511-514 (1934).

TABLE I
PIGMENT CONTENT OF FLOUR EXTRACTS PREPARED WITH NAPHTHA-ALCOHOL
SOLVENT—ALL METHODS

Collaborator	Flour pigment—Samples No.			Method
	1	2	3	
	<i>ppm</i>	<i>ppm</i>	<i>ppm</i>	
3	1.03	1.89	3.10	Spectrophotometric
4	0.94	1.93	3.46	"
5	0.99	1.98	3.14	"
6	1.01	2.13	3.20	"
1	1.05	2.16	3.27	Evelyn photoelectric colorimeter
7	1.05	2.09	3.17	" " "
8	1.10	2.17	3.24	" " "
2	1.05	2.16	3.27	Visual
Mean	1.02	2.04	3.22	
Range	0.16	0.24	0.36	

Equipment

- 3 Bausch & Lomb polarization photometer with Hg vapor light source and filter isolating 435.8 m μ line.
- 4 Bausch & Lomb spectrophotometer with white light source, readings taken at 435.8 m μ , 0.025 mm slit width
- 5 Bausch & Lomb spectrophotometer with Hg vapor light source, readings taken at 435.8 m μ .
- 6 König-Martens spectrophotometer with Hg vapor light source, readings taken at 435.8 m μ .
- 2 Method of Geddes, Binnington, and Whiteside, Cereal Chem. 11: 1-24 (1934).
- 1, 7 & 8 Common standardization—Binnington, Cereal Chem. 17: 639-645 (1940).

TABLE II
PIGMENT CONTENT OF FLOUR EXTRACTS PREPARED WITH NAPHTHA-ALCOHOL
SOLVENT—MEANS OF METHODS

Method	Flour pigment—Samples No.		
	1	2	3
	<i>ppm</i>	<i>ppm</i>	<i>ppm</i>
Spectrophotometric	0.99	1.98	3.22
Evelyn photoelectric colorimeter	1.07	2.14	3.23
Visual	0.95	2.00	3.20

equipment as against the abridged forms employed by Collaborators 3 and 4.

The results obtained by the collaborators using water-saturated butanol extracts are detailed in Table III.

In considering these results, it should be noted that while prepared from the same flours as the naphtha-alcohol extracts, different sample-to-solvent ratios were employed and, therefore, no quantitative relation exists between the two sets of samples. A somewhat greater range is evident in this series of tests even when the results of Col-

laborators 9 and 10 are excluded. This range would be considerably less if the spectrophotometric results obtained by Collaborator 3 were omitted. The best standard for comparison in this series is probably the spectrophotometric results obtained by Collaborator 6. Such a comparison indicates excellent agreement within this group, exclusive of Collaborator 3.

The very low results reported by Collaborators 9 and 10 are apparently due to the use of beta-carotene for calibrating the photoelectric instruments employed and emphasize the importance of a uniform mode of standardization.

TABLE III
PIGMENT CONTENT OF FLOUR EXTRACTS PREPARED WITH WATER-SATURATED BUTANOL—ALL METHODS

Collaborator	Sample			Method
	1	2	3	
	<i>Flour pigment, ppm</i>			
3	1.03	1.94	2.46	Spectrophotometric
6	1.24	2.16	2.57	"
7	1.39	2.26	2.62	Evelyn photoelectric colorimeter
6	1.39	2.26	2.62	" " "
9	.86	1.56	1.87	KWSZ photometer
10	.60	.95	1.10	" " "
3 ^a	1.33	2.27	2.80	Visual (Aminco photometer)
Mean (excluding 9 and 10)	1.28	2.18	2.61	
Range (excluding 9 and 10)	.36	.33	.34	

9 and 10 Calibrated against beta-carotene.

3^a Calibrated against spectrophotometer.

Discussion

The solvents employed for flour pigment determinations extract a complex mixture of pigments comprising alpha and beta carotenes, xanthophyll and its esters, and tricin and other pigments of the flavone type. Since no single pigment is determined as such, the method must be classed as an empirical one. Unquestionably, certain of the individual pigments such as carotene and xanthophyll could be estimated by methods similar to those developed for alfalfa and other feeds containing pro-vitamin A, but no justification is evident for such a course, since the primary object of a flour pigment determination is to secure an estimate of the *total* pigment present as an index of the yellow color of the flour.

In estimating the pigment content of flour extracts by methods other than spectrophotometric, some form of standardization or cali-

bration is essential. This point is of particular importance when photoelectric colorimeters are employed, since some form of filter isolating a narrow region of the spectrum must be used. If such calibration is made with standards differing widely in their spectral properties from those of flour extracts, as is the case with pure carotene, erroneous results are inevitable. It is therefore recommended that flour extracts of known pigment content determined spectrophotometrically alone be employed as a basis for standardizing other techniques.

In the past, the terms carotene and carotinoid pigments have been rather loosely used to indicate the total pigment extracted from a flour by a suitable solvent. In view of the empirical nature of the determination, these terms are entirely too specific and it is, therefore, further recommended that the more accurate term "flour pigments expressed as carotene" be substituted.

Summary

Methods of estimating the pigment content of flour extracts have been submitted to collaborative study in the instance of three extracts and ten collaborators.

Three different modifications of the spectrophotometric procedure, two types of photoelectric colorimeters, and two visual procedures were employed using both naphtha-alcohol and water-saturated butanol solvents.

In general, the results obtained were in good agreement with the exception of two operators using photoelectric instruments calibrated against beta carotene.

The empirical nature of this determination is pointed out and it is, therefore, recommended that the spectrophotometric method alone be employed as a basis for standardizing other techniques.

The use of the term "flour pigments expressed as carotene" is suggested in preference to "carotene" or "carotinoid pigments."

Acknowledgments

The assistance of Mark A. Barmore, R. L. Cunningham, W. F. Geddes, Willis S. Hutchinson, Harold Johannson, R. K. Larmour, V. W. Morris, A. G. O. Whiteside, and W. W. Worzella, who collaborated in this study, is gratefully acknowledged.

THE NICOTINIC ACID CONTENT OF CEREAL PRODUCTS

JAMES M. THOMAS, ALBERT F. BINA, and ELMER B. BROWN

Anheuser-Busch, Inc., Laboratories, St. Louis, Missouri

(Received for publication August 14, 1941)

In a previous communication Bina, Thomas, and Brown (1941) described a procedure for the determination of nicotinic acid applicable to yeast, flour, bread, and cereal products. This method consists essentially in subjecting the sample to be tested to the action of a diastatic reagent (takadiastase) under suitable conditions for diastasis so as to liquefy and saccharify the starchy material in the preparation of the extract. The nicotinic acid content of the extract is then determined after treatment with *p*-aminoacetophenone and cyanogen bromide by reading the color by means of the fluorophotometer. The nicotinic acid content is then evaluated by applying the procedure described in our previous communication.

It was also stated in the previous publication (see "Literature Cited") that other procedures had been studied and found unsuitable for the determination of nicotinic acid in flour, bread, and cereal products because of the semisolid mass resulting from the treatment of this type of material and other interfering products. It was also observed that with the reagents employed, the turbidity and substances other than nicotinic acid influenced the determination to such an extent that the results were erratic and unreliable for our purpose. This was especially true when either aniline, as used by Melnick, Oser and Siegel (1941), metol or acetone, as employed in the procedure of Bandier and Hald (1939), and, in some instances where *p*-aminoacetophenone was employed. It was observed, however, that in the use of *p*-aminoacetophenone no trouble is experienced if the *p*-aminoacetophenone solutions are made up weekly, but may develop with solutions of a month or more of age.

The erratic results obtained when aniline was used as one of the reagents in the procedure may be due in part to the fact that we did not use freshly distilled aniline. In a former research (Nau, Brown, and Bailey, 1925) where aniline was used, erratic results were obtained unless the aniline was distilled daily. Even with this precaution these authors found aniline to be very reactive, giving rise to various anilides if di- or tri-basic acids were present in the reaction mixture.

Experimental

In Table I are assembled the data on a representative sample of a typical Kansas winter wheat mix and on the various milling products

obtained from it in the milling process. These samples were furnished by Mr. Whinery of the Rodney Milling Company. The ash, protein, and moisture were determined by Mr. Whinery and checked by us. The nicotinic acid was determined by the procedure described in our previous communication to this journal, reference to which has been made.

TABLE I
KANSAS WINTER WHEAT MIX

Sample	Ash	Protein	Moisture	Nicotinic acid
	%	%	%	mg per lb
Wheat	1.52	13.00	11.53	6.01
Patent	0.38	11.52	13.68	1.58
Straight	0.43	12.08	13.63	2.03
1st clear	0.67	14.00	13.47	4.15
2nd clear	0.88	14.20	13.30	7.27
Red dog	2.17	16.54	12.58	10.20
Shorts	3.94	17.62	13.00	13.75
Bran	5.89	15.65	13.98	20.43
Germ ¹ (defatted)	3.54	30.90	11.30	18.26

¹ The wheat germ was the only portion that indicated interfering substances of appreciable extent as affecting the analysis. This was noticeable in the orange color tint produced and the abnormally high blanks obtained when the determinations were made without defatting. By defatting the germ with ether, this interference disappeared, giving a normal low blank and characteristic color free from the orange tint. It is our opinion that the lower figure obtained on the defatted material is the correct one for this particular sample and the difference between 24.13 obtained on the original and 18.26 on the defatted material represents the removal of interfering substances rather than the loss of nicotinic acid by the ether extraction. The wheat germ from the spring mix gave the same nicotinic acid content on both the original and defatted material, indicating no loss of nicotinic acid by the extraction, and no interference that was not removed by the blank. The only difference was in the size of the blank values. The ash, protein, moisture and nicotinic acid recorded in the defatted germ column are calculated on the original sample and not on the defatted germ basis.

In Table II are assembled the data on a representative sample of a typical spring wheat mix and the various milling products obtained from it. These samples were supplied by Dr. John Andrews of General Mills, and the nicotinic acid determined in the same manner as with the Kansas winter wheat mix throughout.

TABLE II
SPRING WHEAT MIX

Sample	Ash	Protein	Moisture	Nicotinic acid
	%	%	%	mg per lb
Wheat	1.74	14.10	11.7	9.56
Patent	0.41	12.97	11.4	3.19
1st clear	0.77	16.24	10.7	8.02
2nd clear	2.20	19.71	11.8	8.98
Red dog	3.68	18.78	9.9	14.70
Shorts	5.20	16.79	11.0	17.82
Bran	6.31	16.04	9.9	31.22
Germ (defatted)	4.44	27.53	9.8	28.81

It is apparent that the milling separations used on these two wheat mixes were not identical, and hence not completely comparable for the mill-stream products obtained. However, the wheat samples were ground by us by means of a hammer mill, and the nicotinic acid values obtained are comparable on the wheats. These results show that this spring wheat had 1.59 times the amount of nicotinic acid found in the winter wheat sample. Stated in different terms, the nicotinic acid content of the winter wheat was 62.9% of that of the spring wheat.

In order to check as closely as possible on the applicability of the method, as applied to these products, Dr. Andrews informed us as to the percentages of the total wheat these samples respectively represent. From these percentages and from the amounts of nicotinic acid found in the whole wheat and the various milling products obtained from it, we have calculated the amounts removed by each mill fraction (Table III).

TABLE III
NICOTINIC ACID IN MILL FRACTIONS

Sample	Yield %	Nicotinic acid <i>mg per lb</i>	Nicotinic acid in fractions	
			<i>mg</i>	%
Wheat	100	9.56	9.560	100
Patent	63	3.19	2.009	22.00
1st clear	7	8.02	0.561	6.14
2nd clear	4.5	8.98	0.404	4.43
Red dog	4	14.70	0.588	6.44
Shorts	12.3	17.82	2.191	24.00
Bran	9	31.22	2.799	30.66
Germ	0.2	28.83	0.576	6.31
Total nicotinic acid by calculation of mill streams			9.128	
Total nicotinic acid found by analysis on actual wheat used in the milling			9.560	

The small difference between the total nicotinic acid content calculated from the results obtained by assay of the mill streams and the nicotinic acid found in the whole wheat, indicates that the method of assay is consistently accurate for milling products and cereal grains. It would also indicate that the percentages of the mill streams obtained from Dr. Andrews are quite accurate.

In Table IV are tabulated the nicotinic acid contents of several cereal grains as determined by our procedure. Each of these was ground in a hammer mill through the same mesh screen, care being taken that all passed through the screen.²

² The sample of barley used was ungraded midwestern barley. The malt was No. 3 midwestern malt and bears no direct relationship to the barley sample.

TABLE IV
CEREAL GRAINS

Sample	Protein	Ash	Moisture	Nicotinic acid	
	%	%	%	mg per lb	mg per lb dry solids
Peanuts: raw	25.52	2.23	9.40	43.58	48.11
roasted	24.30	2.21	9.14	45.66	50.14
Wheat mix, spring	14.10	1.74	11.70	9.56	10.83
Wheat mix, winter	13.00	1.52	11.53	6.01	6.79
Barley ¹	10.39	2.48	11.10	6.86	7.72
Malted barley ¹	11.19	2.28	5.63	9.14	9.68
Oats	10.50	3.72	9.39	3.33	3.66
Rye	12.55	1.82	10.47	4.40	4.92
Corn (yellow)	7.50	1.76	14.34	3.17	3.70
Corn (white)	8.87	1.36	12.56	3.35	3.73
Soybean	32.64	5.21	9.99	9.04	10.04
Rice (unhulled)	5.91	4.75	12.27	5.07	5.78
Rice (brown)	7.57	1.05	11.35	3.65	4.11
Rice (polished)	7.22	0.895	12.27	2.099	2.39

¹ The analyses were made on these cereals after they were pulverized in the Wiley Laboratory Mill, Intermediate Model.

The nicotinic acid contents of these cereals were calculated on the moisture content, as received, and also on a moisture-free basis. The results in the moisture-free column therefore afford reliable comparisons of the amounts of nicotinic acid contained in the different cereals.

TABLE V
FLOURS

Sample	Ash	Protein	Moisture	Nicotinic acid
	%	%	%	mg per lb
WHOLE-WHEAT FLOURS				
Purina	1.65	14.00	10.0	6.23
Staff	1.62	15.27	11.7	7.09
Spring	1.75	14.60	11.8	7.44
WHITE FLOURS				
Rodney	0.37	11.35	15.0	1.55
Gibralter	0.39	11.74	15.0	2.86
Spring	0.41	12.86	12.9	3.37
Dulle	0.40	12.00	15.0	3.85

Table V contains the data on the nicotinic acid contents of different flours. Some of the flour samples were obtained from bakeries and are typical of flours used in bread manufacture.

The whole-wheat flours did not show as high a nicotinic acid

content as did the ground whole wheats used. This would indicate that some of the higher nicotinic acid portions of the whole wheat are eliminated in the milling process.

Table VI gives the nicotinic acid content of several samples of bread, including whole-wheat bread, white bread and enriched white bread. The nicotinic acid content is calculated to a 34% moisture basis. The two samples under "white bread" differ only in the flour used in the same bakery. The "enriched bread" samples were purchased on the market in different sections of the country and are representative of the nicotinic acid content of commercial enriched bread. The nicotinic acid content of the enriched white breads is considerably higher than for whole-wheat bread.

TABLE VI
BREADS

	Nicotinic acid <i>mg per lb</i>
WHITE BREAD	
Bread No. 1	1.90
No. 2	2.42
WHITE BREAD (ENRICHED)	
Sample A	8.50
B	9.69
C	9.96
WHOLE-WHEAT BREAD	
Cap sheaf	4.08
Staff	5.25

The nicotinic acid content of yeast is relatively high, but varies somewhat according to culture and environmental conditions. Regular baker's yeast normally contains from 40 to 70 mg per pound of nicotinic acid as received by the baker and approximately $3\frac{1}{3}$ times this amount on a dry basis. Enriched yeast is standardized to contain not less than 500 mg of nicotinic acid per pound. Table VII contains data on the nicotinic acid content of different yeast samples.

Discussion

In the preparation of an extract of a cereal product for analysis it is difficult to determine the completeness of the extraction procedure for bringing the material into solution. We cannot state positively

TABLE VII

YEASTS

	Nicotinic acid	
	<i>mg per lb, 70% moisture basis</i>	<i>mg per lb, dry basis</i>
BAKER'S YEAST		
No. 1	58.5	195
2	57.3	191
3	56.1	187
BAKER'S YEAST (ENRICHED)		
No. 1	549.9	1833
2	558.3	1861
3	561.0	1870

that our extraction procedure removes all of the nicotinic acid from the products we have analyzed, although the results are reproducible on all of the materials analyzed and complete recovery of the superimposed nicotinic acid is obtained. The fact that the total of the nicotinic acid contents determined in the various ingredients used in bread is found to agree with the analysis of the bread itself, leads us to believe the extraction is complete.

We are aware of the possibility of interference from compounds other than nicotinic acid, which are not biologically active but which may produce a color reaction similar to that produced by nicotinic acid in the presence of an aromatic amine and cyanogen bromide. The results obtained lead us to conclude that cereal products do contain such compounds. These compounds are not completely removed by decolorizing the aqueous solution and they may in turn affect the color determination, if the color produced is read in the aqueous extract. We believe that the use of an organic solvent, for example ethyl acetate, as recommended by Harris and Raymond (1939), which will extract the color complex produced by the König (1904) reaction from the other interfering substances that may be present in the original extract, gives a better procedure for eliminating interfering substances than the complicated decolorizing techniques. These complicated decolorizing procedures, as pointed out by Dann and Handler (1941), may, in addition, introduce a source of error due to loss of nicotinic acid through adsorption and elution.

p-aminoacetophenone has proved the most reliable of the aromatic amines. It is more stable, the color complex produced with it is readily and completely extracted by ethyl acetate, and the color is

quite stable to light. None of the other amines tried met these important requirements.

Summary and Conclusions

We have applied our method for the determination of nicotinic acid to a number of cereal products. The method includes liquefaction of the sample by the action of a diastatic reagent, extraction of the vitamin by autoclaving a water suspension of the material, conversion of the starch to sugar, separation and hydrolysis of the extract with dilute hydrochloric acid, adjustment of the pH for proper color development with *p*-aminoacetophenone, cyanogen bromide, and nicotinic acid, extraction and separation of the color complex from extract with ethyl acetate, and the determination of the color in the ethyl acetate phase by means of the fluorophotometer, involving comparison with a known standard of nicotinic acid similarly treated.

Products like wheat germ contain large amounts of material extractable with fat solvents. These products give high blank values and may produce colors that interfere with fluorophotometric readings. These substances can be removed by ether extraction of the original sample. The above procedure eliminates interference from color-producing compounds contained in cereal products that would otherwise produce erratically high results if not eliminated in the nicotinic acid determination.

The results reported give the nicotinic acid content of a number of cereal and baking products. The nicotinic acid content is appreciable and variable in wheat and flour, but considerably lower than the amounts reported by investigators employing other procedures.

The nicotinic acid contents of the various milling products of wheat were determined. The bran was found to contain the highest percentage, and the largest amount of this vitamin of any of the milling products. The percentage in mill products increased as the ash content increased from short patent flour to the bran. The patent and first and second clear flours combined contained approximately one-third and the mill feeds approximately two-thirds of the nicotinic acid of the wheat.

The spring wheat sample contained the largest amount of nicotinic acid per pound (10.83 mg) with soybean second. Oats contained the least amount per pound (3.66 mg) with corn almost the same (3.70 mg). Malt, barley, rye, and brown rice were between these values.

Whole-wheat flours showed less nicotinic acid than ground whole wheats, indicating they had undergone some refinement in the milling process.

The nicotinic acid content of whole-wheat bread, *i.e.* whole-wheat bread made from whole-wheat flour, was above the minimum requirement for enriched bread, but considerably below the average nicotinic acid content found in enriched white bread.

The nicotinic acid content of baker's yeast showed from 55 to 60 mg per pound on a 70% moisture basis, and 190 mg per pound on a dry basis. Enriched baker's yeast showed approximately 550 mg per pound on a 70% moisture basis.

Literature Cited

- Arnold, A., Schreffler, C. B., and Lipsius, S. T.
1941 Chemical determination of nicotinic acid. *Ind Eng Chem. (Anal. Ed.)* **13**: 62-63.
- Bandier, E., and Hald, J.
1939 A colorimetric reaction for the quantitative estimation of nicotinic acid. *Biochem. J.* **33**: 264-271.
- Bina, A. F., Thomas, J., and Brown, E. B.
1941 The determination of nicotinic acid in bread and cereal products. *Cereal Chem.* **18**: 661-666.
- Dann, W. J., and Handler, P. J.
1941 The quantitative estimation of nicotinic acid in animal tissues. *J. Biol. Chem.* **140**: 201-213.
- Harris, L. J., and Raymond, W. D.
1939 Assessment of the level of nutrition. A method for the estimation of nicotinic acid in urine. *Biochem. J.* **33**: 2037-2051.
- König, W. J.
1904 LXIX. Über eine neue, vom Pyridin derivierende Klasse von Farbstoffen. *J. Prakt. Chemie* **69**: 105-137.
- Melnich, D., Oser, B. L., and Siegel, L.
1941 The chemical determination of nicotinic acid in biological materials. Read at spring meeting, Amer. Chem. Soc., St. Louis, 1941.
- Nau, C. A., Brown, E. B. and Bailey, J. R.
1925 Methylene-citric anhydride. The aniline derivatives of citric and aconitic acids. *J. Am. Chem. Soc.* **47**: 2596-2606.

THE EVALUATION OF MALT FOR USE AS A FLOUR SUPPLEMENT ¹

ERIC KNEEN and R. M. SANDSTEDT

Department of Agricultural Chemistry, Nebraska Agricultural
Experiment Station, Lincoln, Nebraska

(Read at the Annual Meeting, May 1941)

The use of germinated cereals for supplementing bread flours has been practiced for many years. Baker and Hulton (1908) described this use as "common" as of that date. These authors, by their proposal of an "enzyme theory" of flour strength, probably were the first to recognize the significance of flour enzymes, both amylolytic and proteolytic, in baking. They postulated that the beneficial effect of malt supplement was due to action of the "starch liquefying enzyme" provided by this means.

During the years following 1908 many investigations have been made of malt supplementation and the manner in which flour diastatic activity influences loaf quality. Space does not permit an adequate review of the extensive literature. The reader is referred to the review paper of Hesse (1935) and to papers by Rumsey (1922), Collatz (1922), Green (1934), Read and Haas (1934, 1936), Landis and Frey (1936), and Hildebrand and Geddes (1940). As summed up by Freeman and Ford (1941) the main reasons for the present extensive use of malt supplement are (1) to increase gas production, (2) to improve the crust color, (3) to increase the moistness of the crumb, (4) to impart additional flavor.

Neumann and Salecker (1908) early observed that increases in loaf volume or in gas production were not closely correlated with the "Lintner value" of malt supplement. Following this observation the belief has developed that the factor in malt supplement responsible for increased loaf volume is the alpha component of malt amylase. Munz and Bailey (1937) found that alpha- rather than beta-amylase was responsible for changes in dough mobility and stimulation in loaf volume. Sandstedt, Jolitz, and Blish (1939) postulated that the improving effect of malt on flour doughs was due to the alpha component of malt amylase. Freeman and Ford (1941) conclude that any method for evaluating malts for flour supplementation must be capable of distinguishing between different alpha-amylase contents.

Practically all the workers have emphasized the value of malt supplement in preventing "yeast starvation" and maintaining gas production, particularly during the "proof" period of bread dough.

¹ No. 287, Journal Series, Nebraska Agricultural Experiment Station.

It is notable that most of the researches have been conducted under conditions where sugar content of the dough could well be a factor limiting loaf volume. Blish and Hughes (1932) showed that a study of baking properties other than those related to gas production in the test loaf could have value only when this gas production is adequately maintained. Further, Sandstedt and Blish (1939) demonstrated that the use of fairly high sugar levels in the baking formula combined with proofing to height essentially eliminated sugar production as a variable influencing loaf volume.

Recently malt supplementation studies (Sandstedt, Jolitz, and Blish, 1939; Geddes, Hildebrand, and Anderson, 1941) have been conducted at high enough sugar levels in the baking formula so that it is improbable that sugar production was a factor limiting loaf volume. It is interesting that even at this sugar level (5%) Geddes, Hildebrand, and Anderson (1941) concluded (p. 57) that "the results of the baking tests clearly indicate that the amylase activity of the malted wheat flours is the only property of any significance in their utility for diastating purposes." These results at high sugar levels suggest that, in addition to the effect on gas production in doughs, diastatic supplements may have an important role in enhancing *gas retention* in the oven.

Recent methods for evaluating malt supplements have been based on the technique of Leatherock, McGhee, and Giertz (1937) for producing flours with constant autolytic diastatic values. Davis and Tremain (1938) modified this method to apply to the standardization of six-hour gas production values. Sandstedt (1938) and Hildebrand and Geddes (1940) report data confirming the value of this type of malt evaluation. These methods reflect only the value of malt supplement for enhancing gas production in a dough and supply no information regarding the possible effect on the "oven spring" indicative of gas retention in the oven.

Apparently there have been few attempts to correlate directly gassing power response from malt supplementation with loaf volume increase, or for that matter malt alpha-amylase activity with gassing power response. Geddes, Hildebrand, and Anderson (1941) observed that when flours were malted to a uniform gassing power level the resulting loaves were of uniform size. Sandstedt (1938) found fair correlation between malt alpha-amylase activity and gassing-power response. However the "alpha-amylase method" used in this investigation was the essentially unmodified Wohlgemuth procedure shown later by Sandstedt, Kneen, and Blish (1939) to be a measure not of the alpha component but of the combined actions of alpha- and beta-amylases.

Since the influence of malt supplement on the gassing power of doughs must be due to added alpha-amylase it seems pertinent to examine the relationships between malt saccharogenic activity, alpha-amylase activity, and stimulation of gas production. Further, additional information is needed regarding the ability of malt supplement to enhance *gas retention* during baking and the relationship of present methods of malt evaluation to this response. In the present investigation the alpha-amylase values reported are specific for that component and baking responses were obtained under conditions in which gas production was eliminated as a variable.

Methods

Alpha-amylase activity: Alpha dextrinogenic activity was determined by the technique of Sandstedt, Kneen, and Blish (1939). In this method an accurate evaluation of the alpha component is achieved by measuring dextrinization in the presence of an excess of the beta component. "Alpha-amylase units" are the number of grams of soluble starch which, under the influence of an excess of beta-amylase, are dextrinized by one gram of malt in one hour at 30°C.

Saccharogenic activity: Saccharogenic activity was determined by the method of Kneen and Sandstedt (1941). In this method maltose production from the action of unmodified malt extract on soluble starch over a 15-minute period is measured. "Saccharogenic units" are the number of grams of soluble starch saccharified by one gram of malt in one hour at 30°C. "Lintner values" were calculated according to the technique of Kneen, Beckord, and Sandstedt (1941): Kneen-Sandstedt units $\times 7.5$ = degrees Lintner.

Beta-amylase activity: The primary object of these studies has been to evaluate the relative importance of saccharogenic activity and of alpha-amylase activity in malt evaluation. Accordingly the amount of saccharification attributable directly to the beta component of malt amylase by itself was calculated infrequently. Where recorded, beta-amylase activity was determined by the method of Kneen and Sandstedt (1941). In this method the amount of maltose production attributable to the alpha component is determined and subtracted from the maltose produced by the action of the unmodified malt extract on soluble starch. The difference represents beta saccharification and beta-amylase units are calculated as the number of grams of soluble starch saccharified by the beta-amylase of one gram of malt in one hour at 30°C.

Gassing power: Gas production in supplemented and unsupplemented flours was measured by the technique of Sandstedt and Blish (1934). The Sandstedt-Blish "pressuremeter" was used in conjunc-

tion with doughs made from 10 g flour, 0.3 g yeast, and water and malt extract sufficient to give an added liquid volume of 10 ml. Results are given as mm of mercury measured after 6 hours' action at 30°C and in most instances are recorded as increases in gas production, *i.e.*, as the gas production attributable to the malt supplement.

Baking procedure: The baking procedure used was essentially that recommended by the A. A. C. C. The actual formula was 100 g flour, 6% sugar, 3% shortening, 1% salt, 3% yeast, and 60% absorption. The same flour was used throughout the baking studies—a commercially milled, unbleached and unsupplemented hard winter wheat flour. The loaves were proofed to a constant height of 9.5 cm. Malt supplements were added to the above formula as desired. In the comparison of different malts a constant increment of 0.1% malt was used throughout. The malts were added as extracts (1 g malt flour plus 100 ml water for one hour at 30°C). Thus a 10-ml portion of malt extract was substituted for 10 ml of water in the formula.

Relationship of Alpha-Amylase to Gassing Power

Since it is more convenient experimentally to supplement the flours with malt extracts than with the malt flours themselves it was necessary to establish the relationship between responses to these two types of addition. Table I shows the results obtained. Two different

TABLE I

COMPARISON BETWEEN THE GASSING POWER INCREASES GIVEN BY MALT EXTRACTS AND EQUIVALENT AMOUNTS OF MALT FLOURS

Malt	Level	Increase in gassing power	
		Flour	Extract
	%	mm mercury	mm mercury
Wheat	0.1	55	53
Wheat	0.2	68	67
Wheat	0.3	82	80
Barley	0.1	57	52
Barley	0.2	77	76
Barley	0.3	85	90

malts (wheat and barley) were used. They were added as 0.01, 0.02, and 0.03 g of malt flour and as extracts equivalent to these weights. It is apparent that extracts produced essentially the same responses as did flours. Accordingly extracts were used in all the following work.

Two types of extracts were used for studying the relationship of alpha-amylase to gassing power: (1) A wheat malt extract heated at 70°C for 30 minutes to inactivate the beta-amylase (Ohlsson, 1930).

This extract had adequate alpha-amylase activity (41.4 units) and no apparent beta-amylase activity. (2) An extract of a hard wheat flour of established freedom from alpha-amylase. The two extracts may then be designated respectively as an alpha-amylase preparation and a beta-amylase preparation. The extracts were added in increments to both an experimentally milled and a commercially milled hard wheat flour² and gassing powers determined. The data are plotted in Figure 1.

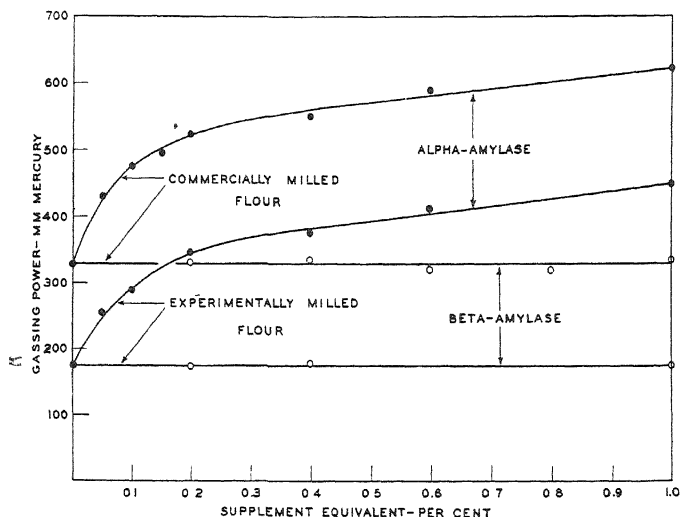


Fig. 1. Relationship of supplementation with alpha- and beta-amylase to the gassing power of flours

As shown in Figure 1 the experimentally milled flour gave lower gassing-power values than the commercially milled. This would be anticipated on the basis of probable content of available starch. Increments of beta-amylase up to the equivalent of 0.1 g flour gave no increase with either flour. On the other hand as increments of alpha-amylase were added gas production was stimulated greatly with the first small increments and then more slowly at higher levels. Alpha-amylase equivalent to 0.02 g malt was sufficient to raise the total gas production of the experimental flour to a level above that of the unsupplemented commercial flour.

The data of Figure 1 supply experimental proof for the supposition that the enhanced gas production of malt-supplemented, over that of unsupplemented, flours is attributable to alpha-amylase activity.

² Neither of these flours was used for the loaf volume and gassing power data obtained with the series of malts and reported under "the evaluation of malts"

Relationship of Alpha-Amylase to Loaf Volume

Three preparations from malt were used as flour supplements in an attempt to isolate the fraction responsible for increase in loaf volume. Unmodified malt extract was used at 0.5% level as a check. This same extract was treated in a manner similar to the Ohlsson (1930) technique for differential inactivation of the two amylase components. Part was heated at 73°C for 30 minutes to provide a beta-amylase-free preparation of alpha-amylase. On analysis it was found that 65.7% of the alpha-amylase activity remained in this extract, while the beta-amylase activity was reduced to zero. The loss of alpha-amylase was greater than is usual with the Ohlsson technique (70°C for 15 minutes) and may be accounted for by the high temperature and prolonged heating used to insure complete inactivation of the beta-component. Another part of the malt extract was acidified to pH 3.3 with hydrochloric acid, held at that pH for 20 minutes at room temperature, then returned to pH 6.0 by addition of sodium hydroxide. This extract retained 77.5% of its beta-amylase activity and only 4.8% of the original alpha-amylase activity.

In the final dilutions of the extracts as prepared for use in baking, the alpha-amylase solution was adjusted so that volume for volume it had the same alpha-amylase content as the untreated malt extract. All three extracts were then used to supplement the baking formula:

TABLE II
RELATIONSHIP OF ALPHA- AND BETA-AMYLASE TO LOAF VOLUME

Malt treatment	Beta-amylase activity	Alpha-amylase activity	Increase in loaf volume	Proof time ¹
	<i>units</i>	<i>units</i>	<i>a</i>	<i>min</i>
Untreated	8.0	50.0	60	46
Heat treated	0.0	50.0	55	47
Heat treated	0.0	40.0	47	46
Heat treated	0.0	30.0	45	47
Heat treated	0.0	20.0	26	46
Heat treated	0.0	10.0	22	47
Acid treated	12.4	4.8	12	46
Acid treated	6.2	2.4	0	46

¹ "Proof time" is the time required to proof the test loaves to a constant height of 9.5 cm.

the untreated extract at 0.5% level, the heat-treated at levels of 0.5, 0.4, 0.3, 0.2, and 0.1% levels. The baking results are shown in Table II. Alpha- and beta-amylase activities are given, treating each increment as if the same volumes of different malts were being used. The loaf volumes are given as increases over that of the unmalted loaf (653 ml) and are averages of two bakes. In addition the average time required to proof the loaves to a constant height of 9.5 cm is recorded. The data of Table II indicate that increase in

loaf volume was dependent on the alpha-amylase content of the extract used for supplementation, thus supporting the conclusion which Munz and Bailey (1937) reached by a similar approach. The hypothesis that small increments of beta-amylase should not influence loaf volume is supported. Also it is difficult to postulate such recorded increases as due to other enzymic activities typical of malt extracts, since these activities (proteolytic, lipolytic, oxidative, etc.) could not be expected to respond to heat and acid treatments in the same manner and degree as does alpha-amylase.

It is obvious that the rate of fermentation during the proofing of the doughs represented by the data of Table II was not influenced by the kind of diastatic supplement. It must be concluded that the rather wide variation in the volumes of the baked loaves was caused by difference in gas-retaining ability during the baking and *not* by variation in gas production during fermentation.

Evaluation of Malts

Malt supplement is usually added as malt flour. Malt extracts are somewhat more convenient to use experimentally and invariably gave more uniform results in duplicate bakes. Table III shows a comparison of the loaf-volume increases resulting from supplementation by six malts added both as flours and as one-hour extracts, all at 0.1% level. Increases given are the averages of four bakes in each case.

TABLE III
COMPARISON BETWEEN LOAF VOLUME INCREASES GIVEN BY MALT EXTRACTS AND EQUIVALENT AMOUNTS OF MALT FLOUR

Malt no	Increase in loaf volume	
	Flour	Extract
	cc	cc
13	49	44
14	45	43
15	40	38
16	34	24
17	29	26
18	27	18

The average unsupplemented loaf volume was 600 cc. The data indicate that the malt extracts were quite comparable to the malt flours in their influence. Increases resulting from the use of extracts were slightly lower in this case than those given by the flours. The data given in Table III for extracts were obtained several weeks after those for the malt flours so the differences in loaf volume increase between the two methods of supplementation cannot be considered significant.

If both gassing-power and loaf-volume increases from malt supplementation are dependent on the alpha-amylase component there should be a very close correlation between such increases and the alpha-amylase contents of various malts. Accordingly the influence of some 24 malts on gassing power and on loaf volume was determined. Extracts were used in all instances and a constant increment level of 0.1% malt for both gas production and baking response. For convenience the malts were separated into three groups: (A) a series of 12 barley malt flours prepared in the laboratory from corresponding malt grains; (B) a series of six commercially milled malt flours including 4 wheat malts, 1 rye malt, and 1 barley malt; (C) a series of six "wheat malt" meals resulting from six different periods of germination of the same wheat sample. Data are given for saccharogenic activity (both Kneen-Sandstedt units and degrees Lintner), for alpha-amylase activity, for increase in six-hour gassing power, and for increase in loaf volume. In addition, the average proof time is recorded for each supplementation.

TABLE IV

RELATIONSHIP BETWEEN SACCHAROGENIC ACTIVITY, ALPHA-AMYLASE ACTIVITY, AND GASSING POWER AND LOAF VOLUME RESPONSES FOR THE TWELVE BARLEY MALTS OF GROUP A

Malt no.	Saccharogenic activity		Alpha-amylase activity	Increase in gassing power	Increase in loaf volume	Proof time
	Lintner value (calc)	Kneen-Sandstedt value				
	<i>deg</i>	<i>units</i>	<i>units</i>	<i>mm Hg</i>	<i>cc</i>	<i>min</i>
1	241	32.1	133.7	142	63	49
2	214	28.5	91.5	120	60	51
3	137	18.3	74.5	100	49	50
4	269	35.9	70.5	97	49	50
5	188	25.0	68.7	102	50	50
6	129	17.2	52.2	79	45	51
7	178	23.7	45.8	75	36	49
8	92	12.2	45.0	73	32	50
9	162	21.6	42.8	73	31	49
10	80	10.7	40.1	72	33	50
11	131	17.4	36.2	67	29	50
12	186	24.8	35.3	67	29	49

The data for the 12 barley malts of group A are shown in Table IV. The malts are listed in order of decreasing alpha-amylase activity. Values given for gassing-power increase are averages of duplicate determinations, those for loaf volume increase averages of from 7 to 8 bakes. It is obvious that there is an excellent correlation between increases in loaf volume and increases in gassing power. Equally striking is the high degree of correlation between alpha-amylase

activity and either of these two increases. On the other hand, the saccharogenic (Lintner) activity of the malt bears little relationship to the value of the malt as a flour supplement.

Times required to proof to the constant height were constant throughout the series and, as in all bakes, were essentially identical with the proof time of the unsupplemented loaf.

Table V lists data for the six miscellaneous malts of group B. In addition to the values for amylase activity and increases in loaf volume and in gassing power, the cereal from which the malt was prepared is given. It should be noted that No. 16 is from the same wheat sample as No. 15, its lower activity being the result of a shorter period of germination. Results given are the averages of duplicate gassing-power determinations and quadruplicate bakes. As with the malts of group A (Table IV), there is a high degree of correlation between loaf-volume increase, gassing-power increase, and the alpha-amylase activity of the malt, and no effect of the malt supplement on gas production during the proof. With this group of malts saccharogenic activity likewise shows a fair degree of correlation with dough response. However, No. 17 is so far out of line that here too, as with the malts of group A, the results indicate that little confidence may be placed in predictions of baking response based on saccharogenic values.

TABLE V

RELATIONSHIP BETWEEN SACCHAROGENIC ACTIVITY, ALPHA-AMYLASE ACTIVITY, AND GASSING POWER AND LOAF VOLUME RESPONSES FOR THE MISCELLANEOUS MALTS OF GROUP B

Malt no.	Malt source	Saccharogenic activity		Alpha-amylase activity	Increase in gassing power	Increase in loaf volume	Proof time
		Lintner value (calc)	Kneen-Sandstedt value				
		<i>deg</i>	<i>units</i>	<i>units</i>	<i>mm Hg</i>	<i>cc</i>	<i>min</i>
13	Barley	155	20.7	65.2	80	44	46
14	Rye	103	13.7	53.5	72	43	45
15	Wheat	68	9.1	31.6	60	38	47
16	Wheat	65	8.6	25.8	47	24	46
17	Wheat	106	14.1	24.3	42	26	46
18	Wheat	47	6.3	22.2	38	18	44

Data on the six germinated wheat samples of group C are given in Table VI. These samples were of a somewhat different nature from usual wheat malts. Sprouts and roots were not removed previous to drying, grinding, and extraction. Sprout length varied from that of No. 24, in which development had just started, to that of No. 19 in which the length was from 30 to 40 mm. The samples are listed in order of decreasing period of germination. Gassing-power and loaf-

TABLE VI

RELATIONSHIP BETWEEN SACCHAROGENIC ACTIVITY, ALPHA-AMYLASE ACTIVITY, AND GASSING POWER AND LOAF VOLUME RESPONSES FOR THE GERMINATED WHEAT SAMPLES OF GROUP C

Malt no	Saccharogenic activity		Alpha-amylase activity	Increase in gassing power	Increase in loaf volume	Proof time
	Lintner value (calc)	Kneen-Sandstedt value				
	<i>deg</i>	<i>units</i>	<i>units</i>	<i>mm Hg</i>	<i>cc</i>	<i>min</i>
19	248	33.0	173.9	141	84	50
20	229	30.5	148.9	135	61	49
21	188	25.1	106.4	110	57	50
22	118	15.7	58.0	92	44	51
23	82	10.9	19.3	43	19	51
24	60	8.0	3.5	1	5	51

volume data are the averages of duplicate and triplicate determinations, respectively. As is customary, the malt extraction was for one hour at 30°C and supplementation was at the 0.1% level.

Gassing-power and loaf-volume data, as recorded in Table VI, show excellent correlation with each other and with both alpha-amylase and saccharifying activities. Greater gassing-power and loaf-volume responses were found for No. 19 than for any other of the malts listed in Tables IV to VI. This sample had the highest alpha-amylase content of all. Likewise, the extract of No. 24 was lowest in alpha-amylase content and gave the least response. On the other hand, these samples were not at the extremes when evaluated on the basis of their saccharogenic activity.

The correlation between loaf-volume response and saccharogenic activity, shown by the malts of group C, must be considered as incidental. During the process of germination not only is there an increase in alpha-amylase activity but likewise a liberation of beta-amylase. Saccharifying power, being the result of the combined action of these two components, is therefore dependent on the period of germination. Since other malt enzymes besides the amylases respond similarly to germination, any attempt to evaluate all malts on the basis of a single set, such as that illustrated in Table VI, might well lead to erroneous conclusions.

Correlation Study

Data obtained for the 12 malts in group A, 5 of the 6 malts in group B, and 1 of the 6 malts in group C were analyzed for correlation. Since Nos. 15 and 16 resulted from different periods of germination of the same wheat sample, only data for sample 15, the higher in activity, were included in the analysis. Similarly, only the data for

sample 19, the highest in activity of those in group C, were included. By this means it was insured that any one malt was independent of all others. It has been pointed out above that evaluation based on a series of malts all produced from the same grain sample (*e.g.*, the six germinated wheats of group C) is inherently of little significance.

The correlation coefficients for the group of 18 malts are given in Table VII. Analysis of the data confirms the conclusions arrived at by inspection. When malt supplementation is used as the only variable, increase in loaf volume and increase in gassing power are both dependent on the increment of alpha-amylase added, and within the limits of the experiment may be attributed to this factor. These two increases, therefore, show a high degree of correlation one with

TABLE VII
CORRELATION COEFFICIENTS BETWEEN THE MALT PROPERTIES INVESTIGATED

Malt property	Alpha-amylase activity	Increase in gassing power	Increase in loaf volume
Saccharogenic activity	+ .7216	+ .7929	+ .7126
Alpha-amylase activity	—	+ .9383	+ .9542
Increase in gassing power	—	—	+ .9351

Note: for 16 degrees of freedom the 1% point is equal to +.590

the other. Saccharifying power is significantly correlated with alpha-amylase activity and therefore with gassing power and loaf volume. This indicates that conditions leading to the development of alpha-amylase activity in germinating grains *usually* influence saccharifying activity in a similar manner. However, no *accurate* prediction of baking response can be made on the basis of malt saccharogenic values either in terms of "degrees Lintner" or any other comparable "unit" of saccharogenesis.

Discussion

The data establish beyond question that alpha-amylase is of significance both in the gassing-power and in the loaf-volume response of flours. This confirms the conclusions of Munz and Bailey (1937), Sandstedt (1938), Sandstedt, Jolitz, and Blish (1939), and Freeman and Ford (1941). It likewise appears that malt evaluation based on the gassing-power technique of Davis and Tremain (1938) is valid, since both gassing-power increase and increase in loaf volume are very highly correlated with the increment of alpha-amylase. If factors in addition to alpha-amylase are influential in the increased loaf volume achieved by malt supplementation, they must be similar to alpha-amylase in their response to acid and heat.

Since both the gassing-power method and the determination of malt alpha-amylase activity are measures of alpha-amylase and thus appear to be equally reliable for evaluating malt, any choice between the two should be based on convenience. Certainly the direct evaluation of alpha-amylase activity is a more accurate and rapid method than indirect evaluation from gassing-power increase. Even assuming that final evaluation is made in the commercial laboratory by determining gassing power or autolytic diastatic response, the malt itself should be purchased on the basis of its alpha-amylase activity. A "high diastatic" or a "strong" malt is not necessarily high in alpha-amylase, the only malt amylase component of any significance for diastating bread flour.

The relationship of alpha-amylase activity to the gassing power of sugar-deficient doughs is readily explained on the basis that the abundance of beta-amylase already present in the flour can saccharify only 60% of the "available" starch of that flour. Alpha-amylase, in combination with the beta component, can carry hydrolysis far beyond that point. In addition, alpha-amylase seemingly is the component of malt responsible for the hydrolysis of "raw," undamaged wheat starch (Kneen, Beckord, and Sandstedt, 1941).

The role of alpha-amylase in baking is obscure. If the sugar level of the formula becomes a limiting factor in fermentation, the sugar produced by the combined action of alpha- and beta-amylase would result in increased gas production and perhaps increased loaf volume. When the sugar level is adjusted, as in the present experiments, in such a manner that gas production is not a limiting factor, loaf volume is dependent upon *gas retention* in the oven. At the present time it appears that one manner in which alpha-amylase action might be effective in gas retention lies in the ability of the enzyme to modify the kind and amount of dextrins in the dough.

Kozmin (1933, a, b) found that in flours of high diastatic activity prepared from sprouted wheat an overabundance of dextrins was produced, this in turn leading to a reduction in bread "quality." Munz and Bailey (1937) noted a pronounced increase in the mobility of doughs supplemented with alpha-amylase and attributed this change to the production of dextrins. Kent-Jones and Amos (1940) found that diastatic supplementation of flour-water pastes markedly increased the "dextrin figure." Freeman and Ford (1941) reported an increase in the dextrin content of bread baked from supplemented flour over that of unsupplemented. Sandstedt, Jolitz, and Blish (1939) concluded that loaf volume stimulation by alpha-amylase was due to its action on the starch and dextrin fractions of doughs. In doughs receiving no malt supplementation there is a considerable

amount of alpha-amylodextrin produced by the action of beta-amylase on "available" starch. Alpha-amylase reduces this dextrin to smaller dextrans and at the same time produces additional dextrans from starch resistant to the attack of the beta-component. Alpha- and beta-amylase acting together rapidly reduce these high molecular weight dextrans to smaller dextrans and sugar. It may well be that at the levels of diastatic supplementation used in the experiments reported above, the increase in gas retention leading to the greater "oven spring" and loaf volume of supplemented flours may be due to such production and *modification* of dextrans.

The intention of this discussion is not to minimize the significance of diastatic supplement in maintaining the gas production of doughs deficient in sugar. Rather it is to point out that "malt response" is a more complex factor than commonly believed. Certainly the data indicate that the ability of a dough to retain gas when baked is influenced by the degree of alpha-amylase activity in the fermenting dough.

Summary

The evaluation of malt for use as a flour supplement has been, and to a minor extent is at present, based on saccharifying power (Lintner value). This saccharogenic activity, though the result of the combined actions of malt alpha- and beta-amylases, is largely determined by content of the beta component. Flour contains an abundance of beta-amylase and consequently could not be expected to respond to the addition of small increments of this component. If amylolytic activity is responsible for malt stimulation of gassing power and of loaf volume, such stimulation must be correlated with the increment of *alpha-amylase* added.

The influence of increments of alpha- and beta-amylase on the six-hour gassing-power values of sugar-deficient doughs was studied. Gas production was not affected by beta-amylase but did respond to additions of the alpha-component. The increase in gas production per increment of alpha-amylase was relatively great for small increments and diminished at higher levels of this component.

Malt extract was treated in such a manner as to give two preparations, one low in alpha- and high in beta-amylase, the other high in alpha- and deficient in beta-amylase. The baking responses of these were compared with that of the original extract. Even though the method of baking eliminated gas production as a variable, in all instances loaf-volume response was proportional to the increment of alpha-amylase added.

Extracts from 24 malts were used to supplement flours and resulting increases in gassing power and loaf volume determined. Both

of these responses were very highly correlated with the alpha-amylase activity of the extract and therefore with each other. Correlation between loaf volume and saccharogenic activity of the malt was significant but could be considered only as incidental and dependent on the tendency for malts of high alpha-amylase activity also to have high saccharogenic activity.

Throughout the wide range of alpha-amylase supplement used in the experimental bakes the times required to proof to constant height showed no significant variation. This demonstrated that malt response under such conditions was *not* dependent on modification of *gas production*.

The data indicate that consideration of alpha-amylase activity is of major importance in evaluating diastatic supplements of flour. Accordingly the purchase of malt supplement should be governed by the content of alpha-amylase, not by the ability of that malt to perform some unrelated function.

It is pointed out that the role of alpha-amylase in modifying the volume of test loaves is complex. In sugar-deficient doughs the role of malt supplement in maintaining gas production is obvious. The present study under conditions in which gas production was not a variable illustrates another manner in which alpha-amylase supplementation influences loaf volume: by modifying the *gas-retaining* capability of the dough during baking. The probable effect of this enzyme on the quantity and quality of the dextrins in dough is discussed.

Literature Cited

- Baker, J. L., and Hulton, H. F. E.
1908 Considerations affecting the "strength" of wheat flours. *J. Soc. Chem. Ind.* 27: 368-376.
- Blish, M. J., and Hughes, R. C.
1932 Some effects of varying sugar concentrations in bread dough on fermentation by-products and fermentation tolerance. *Cereal Chem.* 9: 331-356.
- Collatz, F. A.
1922 Flour strength as influenced by the addition of diastatic ferments. *Amer. Inst. Baking, Bul.* 9.
- Davis, C. F., and Tremain, H. E.
1938 A comparison of methods for evaluating the diastatic properties of malt as a flour supplement. *Cereal Chem.* 15: 826-831.
- Freeman, H. C., and Ford, W. P.
1941 The evaluation of malt products for use in the breadmaking industry. *J. Soc. Chem. Ind. (London)* 60: 6-11.
- Geddes, W. F., Hildebrand, F. C., and Anderson, J. A.
1941 The effect of wheat type, protein content, and malting conditions on the properties of malt wheat flour. *Cereal Chem.* 18: 42-60.
- Green, W. R.
1934 The value of diastatic and other enzymatic activity in flour and doughs. *Cereal Chem.* 11: 319-329.
- Hesse, A.
1935 Über die Verwendung von Enzymen in der Industrie. *Ergebnisse Enzymforsch.* 4: 147-172.

- Hildebrand, F. C., and Geddes, W. F.
1940 Relations between wheat malt dosage, flour diastatic activity, and gassing power. *Cereal Chem.* 17: 626-635.
- Kent-Jones, D. W., and Amos, A. J.
1940 A new flour test: the "dextrin figure." *Cereal Chem.* 17: 265-279.
- Kneen, Eric, Beckord, O. C., and Sandstedt, R. M.
1941 The starch degrading properties of barley malts. *Cereal Chem.* 18: 741-754.
- and Sandstedt, R. M.
1941 Beta-amylase activity and its determination in germinated and ungerminated cereals. *Cereal Chem.* 18: 237-252.
- Kozmin, Natalie
1933a Diastatische Aktivität und ihre Beziehung zur Backfähigkeit der Mehle. *Mühlenlab.* 3: 114-118.
1933b Biochemical characteristics of dough and bread from sprouted grain. *Cereal Chem.* 10: 420-436.
- Landis, Quick, and Frey, C. N.
1936 The enzymes of flour in relation to yeast fermentation. *Cereal Chem.* 13: 281-317.
- Leatherock, L. E., McGhee, H. W., and Giertz, J. W.
1937 Diastatic activity in connection with flour mill control. *Cereal Chem.* 14: 161-181.
- Munz, Emil, and Bailey, C. H.
1937 Effect of the enzymes of malted wheat flour upon certain properties of flour and dough. *Cereal Chem.* 14: 445-457.
- Neumann, M. P., and Salecker, P.
1908 Über den Einfluss diastasereicher Malzpräparate auf die Backresultate. *Landw. Jahr.* 37: 857-872.
- Ohlsson, Erik
1930 Über die beiden Komponenten der Malzdiastase, besonders mit Rücksicht auf die Mutarotation der bei der Hydrolyse der Stärke gebildeten Produkte. *Z. physiol. Chem.* 189: 17-63.
- Read, J. W., and Haas, L. W.
1934 Baking quality of flour as affected by certain enzyme actions. *Cereal Chem.* 11: 280-298.
1936 Further studies on the baking quality of flour as affected by certain enzyme actions. *Cereal Chem.* 13: 14-37.
- Rumsey, L. A.
1922 The diastatic enzymes of wheat flour and their relation to flour strength. *Amer. Inst. Baking, Bul.* 8.
- Sandstedt, R. M.
1938 The relation of alpha- and beta-amylases to the evaluation of malts in flour technology. *Cereal Chem.* 15: 832-835.
- and Blish, M. J.
1934 Yeast variability and its control in flour gassing power tests. *Cereal Chem.* 11: 368-383.
1939 The question of sugar levels in laboratory baking. *Cereal Chem.* 16: 36-42.
- Jolitz, C. E., and Blish, M. J.
1939 Starch in relation to some baking properties of flour. *Cereal Chem.* 16: 780-792.
- Kneen, Eric, and Blish, M. J.
1939 A standardized Wohlgemuth procedure for alpha-amylase activity. *Cereal Chem.* 16: 712-723.

A MICROSCOPIC STUDY OF BREAD AND DOUGH

MERTON E. BURHANS and JOHN CLAPP

Research Department, Petersen Oven Co., Chicago, Illinois

(Received for publication June 16, 1941)

The microscopic components of bread are well known but their physical relationships have usually been surmised by indirect means. This direct microscopic study of bread and its dough entails (1) a review of microscopic structural studies, (2) the devisal of a practical sectioning technique that was used on (3) a series of samples taken from bread, both during and after its preparation, (4) a microscopic examination of the structures and their changes, and (5) some theoretical inferences based upon the foregoing. This report presents the progressive changes during breadmaking, as well as the minute structures themselves.

Many microscopic studies of breadstuffs are concerned with the individual bread components, such as yeast, starch, or other identifiable substances. However, only a few studies have pictured or described the physical relationships of the components. One of the earlier ones was the work of Scheffer (1916). This was illustrated with photomicrographs. Another early study was the article by Verschaffelt and van Teutem (1915). This work has been referred to and reproduced by J. R. Katz in several subsequent articles. Their illustrations are camera lucida drawings from sections of bread. They described the gluten structure in which starch and yeast are embedded, elongation of the starch grains adjacent to air cells, lines of flow similar to those seen on a cut steel surface, and some other phenomena which were attributed to staling changes. A third structural study was made in 1930-31 by Joseph Stagman, Chicago, Illinois. This has not appeared in the literature. He worked with white, rye, and wholewheat breads. The sections were made from washed gluten, sponge, dough, and the bread. In addition to the gluten framework for starch and yeast, he describes the gas pockets and their enlargement during fermentation and baking. Butterworth and Colbeck (1938) published several photomicrographs made from both bread and dough preparations. They described the effect of stretching upon sponge and dough, a "squeezing-out" of the starch from the gluten mechanically produced by leavening, as well as the gluten meshwork in which are embedded the yeast and starch. Each of these studies centers around the

methods used in producing the sections. However, they all agree as to the function of the gluten.¹

Sectioning Technique

Microscopic preparations for showing progressive changes must disclose representative cross sections of the original specimens. The technical difficulties are twofold, thinness for clarity and the prevention of visible changes. The latter is especially troublesome in bread-stuffs because there are no cell walls, skeleton, or analogous structures as in the usual histological material. The gluten acts as a framework, but since its several fractions are soluble in various solvents, the common ones cannot be used. Slight mechanical manipulation inordinately changes the microscopic picture, while processes incidental to preparation often cause physical and chemical changes of a fundamental nature. Thus alcohol, water, ether, and xylol cannot be used; teased material, films, and smears show extensive deviations from the original structure; fermentation and enzymatic action must be completely inhibited; and even the effect of temperature and moisture must be fully considered.

The second difficulty lies in making the preparations thin enough to give a clear microscopic picture. The higher the magnification the thinner these must be made. Those which are too thick appear on the photographic plate as a confused mass in which little can be discerned. For magnifications of 500X or more, the preparations should be 10 microns or less in thickness. Besides those previously mentioned, frozen sections are impractical because of their thickness. Embedded specimens can be cut into thin sections which are adequate. These and other considerations prevent the use of many standard procedures and make the problem of true structural representation a difficult one.

The method of producing the accurate cross sections of bread and dough described herein will be detailed later in a separate article. The highlights of this method are the use of CO₂ ice to stop fermentation, enzyme action, and aging; simultaneous *in toto* fixation and staining by osmic acid vapor; dehydration by CaO; embedding with paraffin; cutting on a sliding microtome; albumen fixation of the sections to the slide; staining as desired; and finally mounting in balsam. Samples of sponge, dough, bread, and the ingredients are all successfully sectioned by this method. The resulting sections can be made

¹ During the preparation of this report two other articles have appeared describing pertinent facts and conclusions. Baker (1941), working with highly oxidized doughs, prepared photographs of films of excessively proofed bread. His conclusion, confirmed by the failure of iodine to stain the surface of the gas pockets, was that there is a glutinous lining of these pockets. Baker and Mize (1946), utilizing macroscopic methods, conclude that the entrained gases, the yeast organisms, and manipulations such as punching and molding are of no consequence as the origin of the gas cells. It further concludes that the gas cells arise in the gluten. Material to be presented in this article corroborates these conclusions by visual evidence.

8 microns or less in thickness and stained with specific stains so that the fats and fatty acids are black and the starch is blue. A variety of differential stains distinguish the acidophilic gluten proteins from the basophilic yeast cells. The combination of red yeast cells and yellow gluten produces a striking picture with the blue starch and black fats.

This technique was applied to a progressive series of samples from a simple white dough. For simplicity, a straight white dough of the following composition was used:

Flour	100%	Corn sugar	4%
Water	62%	Salt	2%
Powdered skim milk	4%	Yeast	2½%
Lard	½%	Malt	½%

The dough was mixed 7 minutes at 56 rpm, scaled and rounded immediately, allowed 30 minutes on the bench, made up by hand, proofed at 90°F for about 55 minutes, and baked 28 minutes in a brick peel oven. The dough schedule and corresponding samples were as follows:

Schedule (min)	Sample	Sample No.
	Ingredients	As marked
0	Just out of mixer	1
10	Rounded, 10 min on bench	2
30	Rounded, 30 min on bench	3
30	Immediately ff makeup	4
40	10 min in proof box	5
85	To oven ff 55 min proof	6
160	Bread after cooling	7

The presentation of sections begins with the ingredients and follows in the order of the samples as listed.

Microscopic Findings

The microscope was a Carl Zeiss medical-type instrument. A 8× ocular was used with 3×, 8×, 40×, and 100× oil-immersion objectives. The camera was a 35-mm focal-plane shutter type. The magnifications given below are purely relative. This stain was a combination of carbol-fuchsin and Bismark Brown, unless otherwise stated.

The flour used in the preparation of the dough was a short-patent hard-wheat commercial variety. Figure 1 indicates the nature of flour granularity. The particles vary in size from individual starch grains to a group of 20 or more cemented together by a matrix of gluten. Since this is a cross section, the individual particles of flour have more starch grains than can be seen in the pictures. Likewise, the average diameter of the flour particles is larger than is apparent,

because the knife of the microtome is more likely to cut through a smaller portion than at the widest part. The large particle of flour in Figure 2 shows no systematic arrangement of starch. Figure 3 brings out the typical structure of wheat starch; the small grains are nearly spherical; the intermediate ones, ovoid; and the large ones,

FLOUR



Fig. 1. Flour: a, large particle; b, small particle. 64X.

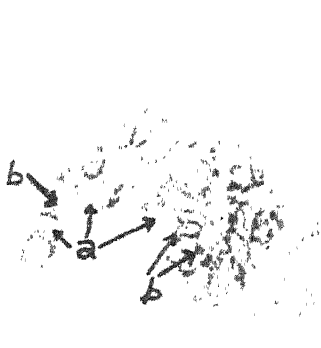


Fig. 2. Flour: particle a, starch; b, gluten matrix. 320X.

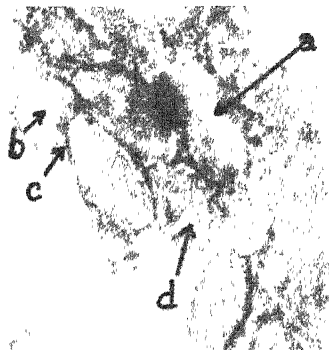


Fig. 3. Flour: a, edge of large starch grain; b, small spherical grain, on surface; c, gluten on surface; d, hilum of grain. 800X.

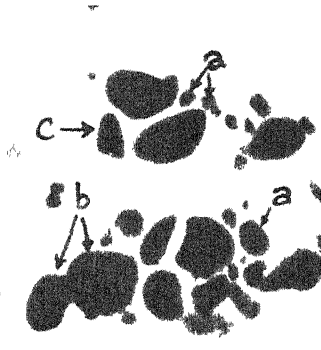


Fig. 4. Flour: (deep iodine stain) a, small starch grain; b, large starch grain; c, broken grain. 800X.

either elliptical with the median "crease" (*i.e.*, edge), or roughly ovoid, depending upon the aspect presented. The starch lies in contact with the gluten matrix, yet well defined from it by a smooth, uniform, fine line. Figure 4 was deeply stained with iodine (Lugol's solution) causing the starch to stain an opaque blue-black. The starch grains vary widely in size and shape; two broken ones are shown.

Powdered skim milk (Fig. 5), roller process type, shows a longitudinal cross section through a large flake. Figure 6 shows the same, except that it has been cut across the flake. Figure 7 is a higher magnification of Figure 5. Figure 8 shows an unstained flake. Powdered milk cannot be identified when mixed into dough as it has no characteristically formed elements and no staining reaction.



Fig 5. Milk flake 64X.



Fig 6 Milk, cut across the flakes 64X

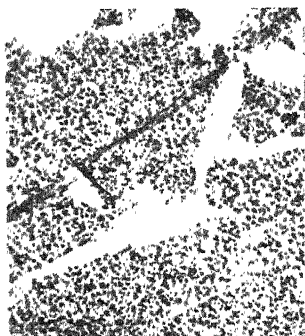


Fig 7 Milk flake 320X



Fig 8 Milk, unstained 800X.

Lard was run through the same preparatory technique as the other samples and Figures 9-11 were obtained. In relatively large amounts it does not show formed elements nor its characteristic staining reaction, and consequently has an entirely different appearance in bread-stuffs.

The first dough sample was taken directly from the mixer. Figure 12 shows a confused mass of starch and the darker gluten. There are

no gas bubbles present and no order or alignment of the starch and gluten is apparent. Figure 13 shows an enlarged view of the same and brings out the lack of organized structure. In Figures 13 and 14, and with the other high magnifications of dough, small irregular spaces are seen which are artifacts resulting from the shrinking of the gluten away from the starch, due to dehydration during preparation. Both figures also show, evenly dispersed in the gluten matrix, small, dark,

LARD

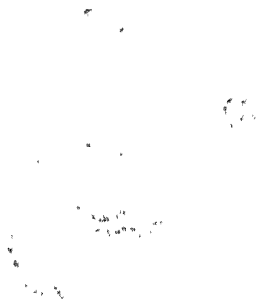


Fig. 9. Lard. 64X.



Fig. 10. Lard. 320X.

Fig. 11. Lard. 800X.

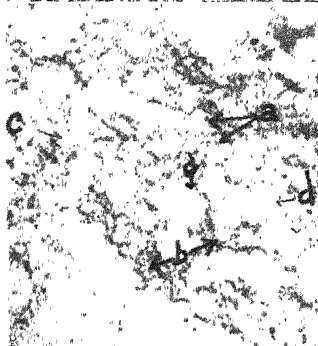
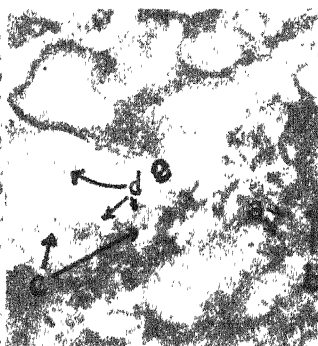
oval areas, which are deeply stained yeast cells. Fat stains a deep brown or jet black, but throughout the sections of dough there are no black areas which approach even the size of the yeast cells. But, wherever the edge of starch granules may be clearly discerned, there is often a black beading along the periphery, as may be seen in Figure 14. Since these are not seen in flour (Fig. 3) and the black stain is a specific stain for fat, this may be the dispersed shortening.

The second sample of dough was taken 10 minutes after mixing. Figure 15 shows two small gas bubbles which are the beginnings of leavening as produced by yeast fermentation. Some concentric axial alignment of the starch and gluten may be seen just peripheral to each bubble, while the intervening dough shows none. Figure 16 is an enlarged view adjacent to a bubble. This picture shows the even

DOUGH—SAMPLE 1



Fig. 12 Dough 64X

Fig. 13. Dough: *a*, yeast cells; *b*, gluten; *c*, starch; *d*, spaces (artifacts). 320X.Fig. 14. Dough: *a*, yeast cells; *b*, gluten; *c*, starch; *d*, black beading; *e*, spaces (artifacts). 800X.

dispersion of the yeast bearing no relation to the bubble. Figure 17 is an enlarged view of Figure 16 showing a very small sector of the edge of a gas bubble. As can be seen, both gluten and starch form its walls. The matrix conception of gluten is quite evident, showing large and small starch granules as well as yeast, dispersed in a gluten background. Again the black dots on the periphery of the starch granules are evident.

Sample 3 had 30 minutes on the bench, being ready for makeup. The principal way in which Figure 18 differs from the corresponding Figure 15 (sample 2) is the presence of more and enlarged gas bubbles. Figure 19 shows both gluten and starch forming the gas bubble wall. Figure 20 shows black beading on surfaces of gluten where it has shrunk away from the starch as well as on the starch. The findings are otherwise the same as before.

SAMPLE 2

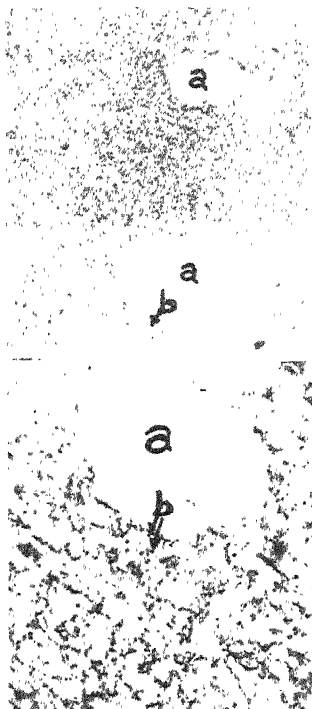


Fig. 15. Dough: a, gas bubbles; b, slight concentric changes. 64X.



Fig. 16. Dough: a, small gas bubble; b, yeast. 320X.

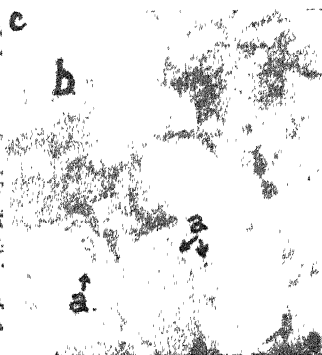


Fig. 17. Dough: a, typical peripheral beading; b, gluten matrix; c, gas bubble. 800X.

Sample 4 was taken after the dough was made up by hand. Figures 21 and 22 demonstrate alignment of the starch granules and gluten other than concentrically around the gas bubbles. There are many groups of several large starch granules which are seemingly arranged in layers. The gluten appears as strands which send off interlacing fibrils to separate the starch. The gas bubbles are deformed and fewer, though not absent, as may be accounted for by the hand makeup

which does not expell all the gas. Figure 23 demonstrates that the gluten is not actually in strands and fibrils, but that there is segregation of gluten and starch. The larger grains of starch are segregated much more than the smaller, which, as well as the yeast, remain embedded at random in the aggregated gluten. Figure 24 (high magnification) shows nothing not previously demonstrated, but it does show

SAMPLE 3

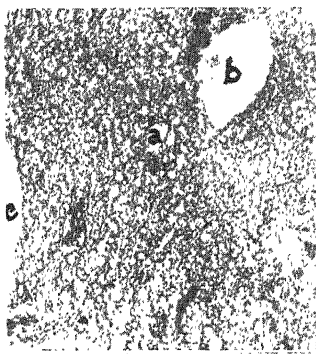


Fig 18 Dough *a*, small gas bubbles, *b*, medium gas bubbles, *c*, large gas bubbles. 64 X

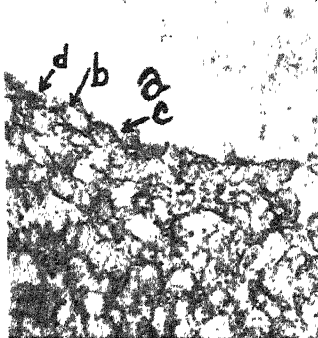


Fig 19 Dough: *a*, gas bubble; *b*, alignment of starch, *c*, gluten portion of bubble wall, *d*, starch portion of bubble wall. 320 X

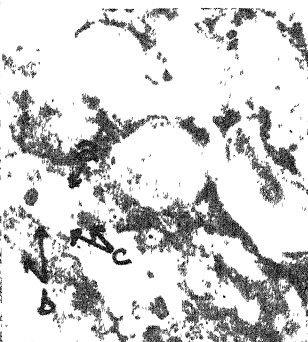


Fig 20 Dough *a*, peripheral beading, *b*, beading on shrunken gluten surface; *c*, comparative sizes of yeast and small grains of starch. 800 X

the presence of numerous specks of dust. It is significant that the above changes appear after the process of makeup.

The dough for Sample 5 was taken after 10 minutes of proofing, following the hand makeup. The three figures show no changes other than more gas bubbles and their enlargement. Figure 25 shows rather well (at "*c*") stratification and aggregation of the starch and gluten.

Figure 26 also illustrates this and clearly presents the wall of a gas bubble. This picture is typical of all of the walls of the gas bubbles throughout the dough samples. Both starch and gluten form the surface of the wall. There is the characteristic concentric alignment of the starch axes, while together the starch and gluten form the con-

SAMPLE 4

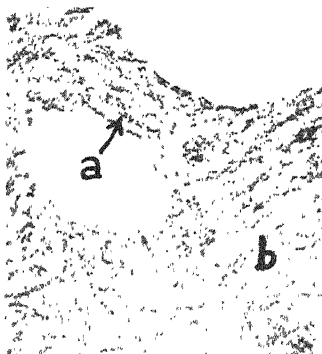


Fig. 21 Dough lightly stained: *a*, concentric alignment; *b*, stratification and grouping. 64X.

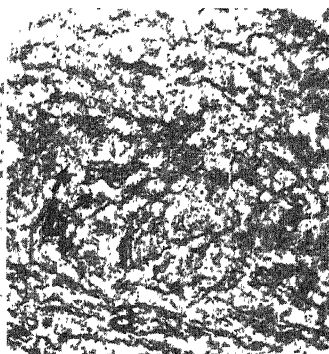


Fig. 22 Dough, gluten deeply stained: *a*, gluten stratification ('gluten strands'); *b*, groups of starch. 64X.

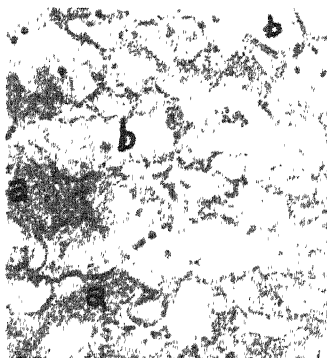


Fig. 23 Dough: *a*, aggregated gluten; *b*, groups of starch. 320X.

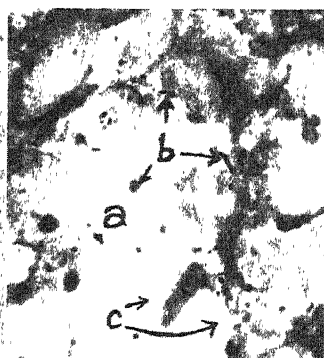


Fig. 24 Dough: *a*, shrinkage space; *b*, dust artifacts; *c*, beading. 800X.

centric strata, as previously mentioned under Sample 2. In Figure 27 some of the starch granules show distinct peripheral beading, indicating fat; the yeast shows particularly well, while the gluten appears somewhat granular.

Sample 6 was taken 55 minutes after being made up, at which time the loaves were judged ready for the oven. Figure 29 (also Fig. 28, under Sample 7) demonstrates that the structure is changed from a more or less solid dough mass with an occasional gas bubble to a porous one of numerous large and small gas pockets with walls of intervening dough (the "air cells" of the bakers). The gluten and

SAMPLE 5

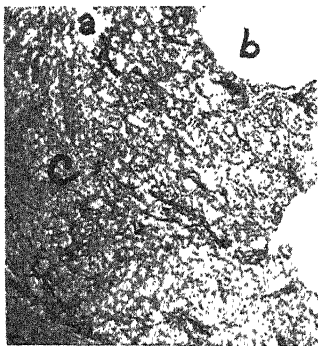


Fig. 25. Dough: *a*, early bubble; *b*, bubbles; *c*, intervening dough structure. 64 \times .

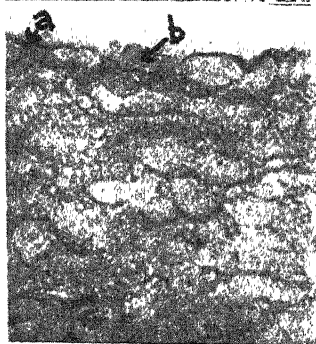


Fig. 26. Dough: *a*, gluten lining of bubble; *b*, starch wall. Note horizontal alignment of starch axes and "layers" of gluten. 320 \times .

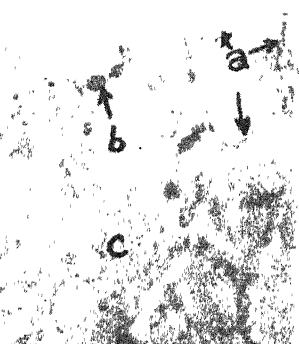


Fig. 27. Dough: *a*, beading; *b*, large yeast cell; *c*, gluten. 800 \times .

starch arrangement shows that the concentric alignment characterizes a much greater part of the dough structure at this stage. Figure 30 was taken at the junction of three gas pocket walls and shows no deviations of structure. Figure 31 shows no changes from previous samples.

The specimens for Sample 7 were taken from the cooled bread crumb. The gas cells are so large and numerous that their comparison

before and after baking is possible only with low magnification. Comparison of Figures 28 and 32 (dough just before baking and bread just after, respectively) indicates that baking causes enlargement of the gas cells, an increase in their number, marked thinning of the cell walls, and a sharp increase in the number of intramural gas pockets.

Figure 33 is bread under the same magnification as the low powers for previous samples. The moderate-sized gas cells and intramural

SAMPLE 6



Fig. 30. Dough (poor focus): a, gas cells; b, random yeast dispersion. 320 X.

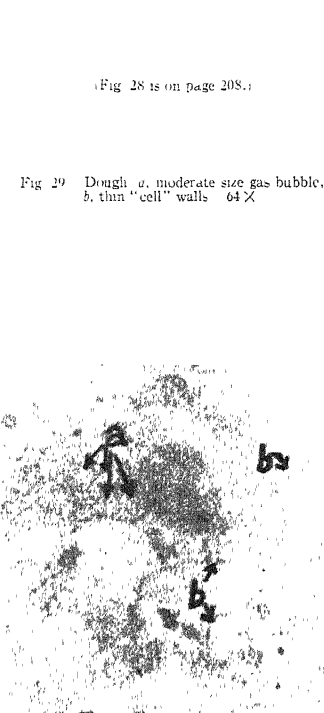


Fig. 31. Dough: a, small embedded starch grain; b, peripheral beading. 800 X.

pockets are shown. At "c" is an area which represents a longitudinal section of wall between two gas cells, hence tangential to them. The starch is nearly transparent, while the deeply stained gluten and yeast (the fine black dots) outline it. The concentric stratifications remain around the gas pockets, while the intervening walls show that these extend outwards to merge imperceptibly with the next or confluent change direction in the tangential area to correspond to each other.

Figure 34 shows the junction of a very thin and a moderately thin wall. In the thinner one, starch is the major structure, the gluten being extremely thin or perhaps even absent. The starch at "a" shows extreme elongation while at "c" very little change is apparent.

BREAD—SAMPLE 7

Fig 28 Dough (Sample 6): *a*, large gas cell, *b*, medium gas cell, *c*, intramural gas pocket, *d*, cross section of typical cell wall. 24X

Fig 32 Bread (Sample 7): *a*, gas cells; *b*, intramural gas pockets, *c*, cross section of cell walls. 24X

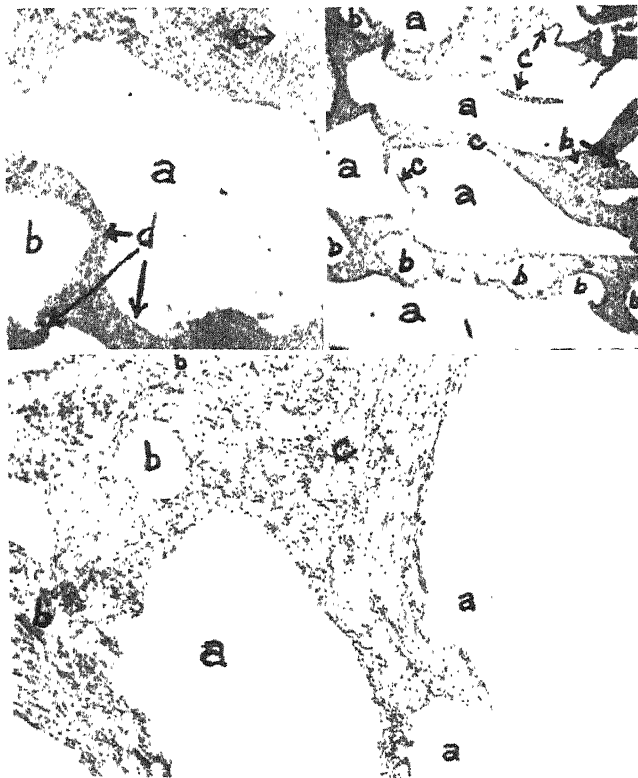


Fig. 33. Bread: *a*, medium sized gas cells; *b*, intramural gas pockets; *c*, tangential cell wall. 64X.

Figure 35 shows one of the thicker cell walls. At "a" there is a group of swollen but individual grains of starch with no material between them. Many of the starch grains (as at "c") appear somewhat swollen but otherwise unchanged, even retaining the faint crease of the

edge. At "b" and other places are black areas of irregular shape, varying in size up to that of a small starch grain. These black areas demonstrate the specific staining reaction for fats. The apparently unchanged yeast cells remain at random in the gluten matrix. Both Figures 34 and 35 show that the gas-retaining walls are surfaced with gluten and starch.



Fig. 34. Bread thin cell wall *a*, "stretched" starch, *b*, gluten of thin wall, *c*, "static" starch, *d*, starch and gluten surface. 320X.

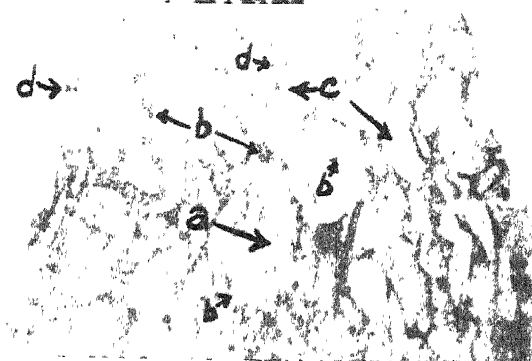


Fig. 35. Moderate cell wall *a*, swollen starch, *b*, specifically stained fat, *c*, apparently unchanged starch, *d*, yeast cells. 320X.

Figure 36 is a picture of a typical moderately thin cell wall. The fat, as seen at "b" and other places, occurs in variously sized droplets from very small globules to irregular masses several times the area of a yeast cell. The larger lipid masses are in contact with the starch while the smaller globules may also occur in the gluten. This picture is otherwise an enlarged view of the structural features presented under Figures 34 and 35.

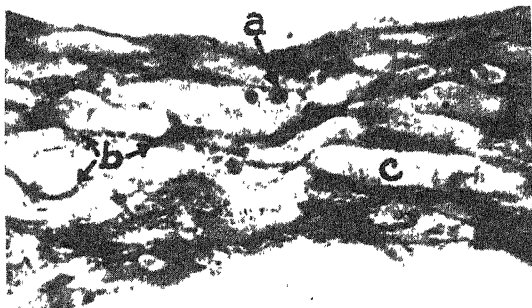


Fig 36. Bread, cell wall *a*, yeast cell showing nucleus, *b*, specifically stained fat, *c*, starch, *d*, gluten 800 X.

Discussion

The foregoing illustrations were made sequentially to show, where possible, the structural changes in the material during its manufacture. Causes and effects are discussed under the headings (1) effects of mixing, (2) effects of processing, and (3) changes during baking. While osmic acid was used primarily as a fixative, it coincidentally and selectively stains the lipid substances. This feature makes possible a study of (4) the role of shortening. Immediate freezing of the samples and their fixation without thawing accurately preserves the microscopic features and consequently enables a logical hypothesis of (5) the mechanism of yeast leavening and the rational (6) correlation of microscopic structures with crumb quality. These and other preliminary studies did not reveal changes in the dough substance directly ascribable to fermentation nor show changes in bread attributable to staling. This does not mean that such are absent, but that further critical studies are indicated.

Effect of mixing: Comparison of the flour and first dough sections (Figs. 1-4, 12-14) demonstrates that during mixing the particles of flour lose their individuality. Furthermore, the even dispersion of the yeast indicates the breaking down and *complete* dissolution of these particles. The total absence of obvious lipid masses in the dough shows that the shortening is colloiddally dispersed at this early stage. In summation, mixing produces a homogeneous suspension of starch, fats, and yeast in the glutinous medium constituting the dough.

Effect of processing: The sequence of dough samples illustrates changes referable to the intermediate proof, molding and the prebaking proof. As anticipated, generalized alterations are not microscopically demonstrable as a result of cutting, scaling, or rounding. Gas pro-

duction first becomes apparent in the second dough sample (Figs. 15-16). As a result, tension occurs in the matrix just peripherally to each gas pocket and produces the tangential alignment of the long axes of the starch. This has been described by Katz and others and apparently always accompanies gas production. Sample 3 indicates further formation of gas by an increased number of variously sized pockets as well as by simple enlargement.

That molding (Sample 4) knocks out much of the gas is evidenced by reduced number and by irregularity of the spaces. The arrangement of the starch is not lost but is extended generally throughout the mass by the coincidental stresses longitudinal to the resulting sheet of dough. Through the "squeezing-out" mechanism of Butterworth and Colbeck (1938), the starch grains tend to segregate into groups of about 10 to 15 each. These groups are made predominantly of the larger grains and evidently are an important consequence of molding (*i.e.*, "mechanical development"). This conversely coalesces the remaining matrix into sheets which appear in section like strands (Figs. 21-22) entwined between the starch. The known improvement in the dough and the overlapping of these sheets are direct evidence of the resulting better gas retention. Following molding there ensues a prolonged period of leavening in which the mechanisms remain the same but the picture becomes remarkably altered. At this time gas pockets reappear in increased numbers and enlarge far beyond previous limits. The impression changes from one of entrapped bubbles in the dough to the porous, honeycomb structure of Sample 6. The apparent strands of the intervening walls (Samples 5 and 6) become confluent. The walls and the gluten are obviously thinned by extension. There is little evidence indicating that rupture is the principal means of bubble enlargement.

Changes during baking: After dough fermentation, the action of heat in baking soon produces crust formation and a rather sudden volume increase, while later the entire mass becomes relatively dry and rigid. Underlying these gross changes are the alterations of the minute structures, including the starch, shortening, gluten, and gas pockets. The oven-spring results from three factors: first, faster gas production as discussed separately; second, further extension subsequent to both the softening of gluten by heat and plasticization of the starch; and third, the rapid appearance of numerous new bubbles in the walls of the pre-existing ones (Figs. 28 and 32). Increased extensibility is evidenced by the remarkable thinness of the bubble walls and of the gluten "strands." Plasticity of the starch is shown by its conformity and elongation as part of the walls. Since this is a stage of gelatinization, it is coincidental with the dehydration and fixation of the gluten. Figures 33, 34,

and 35 attest that wheat starch neither ruptures nor coalesces while gelatinizing the outlines of individual grains, their "creases" and freedom from spherical masses demonstrate this. The altered distribution of the lipids indicates that coalescence takes place as described later. Visual changes are not shown in the coagulated protein substances, *i.e.* the gluten and yeast.

The role of shortening: Frey and Landis (1932) express the commonly held view and cite literature to the effect that shortening has lubricating action in the dough and is related to surface tension in some poorly understood fashion. The presented series indicate that not only are both probable, but that both are expressions of the same underlying phenomenon. The lipids of dough are derived from the flour and from the ingredients added at mixing, notably the shortening. Flour extractions yield 1.3% or more fatty substances, varying with the flour fraction, and based on methods which are known to only partially extract the lipids (Sullivan, 1940). The microscopic examination (not shown in the pictures) of flour reveals occasional minute black deposits of stained lipids lying in the protein matrix. These are smaller than the smallest starch grains and may or may not lie in contact with them. The picture becomes very different in the dough samples. The pictures from Samples 1 to 6 show the absence of the black deposits, frequent spaces between the starch and gluten, and a fine black beading at the edge of the starch grain, or on the edge of the shrunken gluten. The absence of stained fat deposits appears significant because more lard (4%) than yeast (2.5%) was added to the flour, yet the yeast is easily identified as oval bodies 5 to 7 microns in diameter, while the lipids are not, despite their specific staining reaction. The possibility of a faulty technique in dissolving out the fat is contraindicated by the presence of the staining reaction in both flour and bread, and by the sample of lard which was taken through precisely the same preparation.

One can conclude, then, that the dispersion of the lipids in dough is a very fine one, perhaps bordering upon the ultramicroscopic. The last two findings (*i.e.*, spaces and beading) are presumptive evidence that the shortening is spread on the interfaces between the relatively dry surface of the crystalline-like starch grains and the moist gluten. The beading is direct visual evidence while the spaces indicate that something interferes with the adhesion of the gluten and starch. Furthermore, the fineness of dispersion on the starch surface is the natural consequence if the 0.04 g of lard per gram of flour (as used in formula) even partially covers the 2,004 sq cm of starch surface as calculated by Stamberg (1939).

The indirect and negative evidence also suggest that the shortening of the dough is dispersed upon the starch surface. The impenetrable

nature of both the yeast cells and starch grains renders unlikely any dispersion within their substance. The gluten matrix is a moisture-bearing colloidal system that has little affinity for hydrophobic substances such as lard. Moreover, the bread sections do not fulfill the anticipated picture that would result from a suspension of fats in the gluten matrix. In such a case, the bread and dough sections might manifest deposits of lipids within the gluten; if the actual dough sections represent a fine gluten dispersion, the bread sections should retain the beading along the starch, the spaces between the starch and gluten, and should not reveal the obvious collections of lipids in contact with the gelatinized starch.

In contrast, the presented sections better fulfill the original premise. During baking, the fat coalesces into larger droplets that bear relation to the starch because of the previous surface dispersion. This coalescence is a rational consequence of gelatinization which, by water absorption, alters the capillary forces acting on the fats. The running together of the fats removes the interposing material between the gluten and starch. The resulting adhesiveness is shown in the pictures by the absence of the starch-gluten spaces. Finally, these considerations rationally correlate the large, specifically stained, lipid deposits that appear in the bread sections, the other microscopic observations, and the physico-chemical properties of the components. Briefly, the shortening is dispersed upon the interfaces of the starch and gluten within the dough, but coalesces into droplets during baking.

Mechanism of leavening: Yeast contains, among other enzymes, intracellular zymase which breaks down glucose to CO_2 and alcohol.

As stated, this action is intracellular and not diffused through the dough, so one anticipates gas bubble formation to bear some direct relationship to the position of yeast cells. However, the microscopic findings show that practically all of the yeast is embedded, at random, deep within the gluten matrix.

The CO_2 formed within the yeast cell diffuses through the cell membrane into the surrounding moisture-containing suspension, where at first it remains in solution. In physico-dynamic terminology, the cohesiveness of the gluten is greater than the vapor pressure of the CO_2 in solution. By the laws of diffusion the CO_2 pressure tends to equalize itself, but since the CO_2 is continuously produced, there is a decreasing pressure gradient radially from each yeast cell. This gradient is more complicated than in a simple suspension because the starch contains only a little "bound" water, and consequently the CO_2 diffuses around these granules. However, where several of these gradients meet there forms an area of higher vapor pressure which lies between the several yeast cells, but, in cross section, bears no apparent

relationship to them. When the vapor pressure exceeds the cohesiveness, the gluten gives way at its weakest point within the high pressure area. Mechanically a group of starch granules with little intervening gluten forms a weak point and simultaneously serves as a nucleus for gas formation. As the gluten gives way, gas pockets form. Continued CO_2 production enlarges existing bubbles by diffusion and the resulting tension raises the vapor pressure to form others.

During baking the mechanism becomes complicated by the several effects of temperature. Its progressive rise renders the CO_2 less and less soluble, causes its expansion, and so affords continuous leavening action through most of the baking process. Initially the rising temperature also stimulates zymase action so that at $50^\circ\text{--}55^\circ\text{C}$ (beginning gelatinization of wheat starch—Herter, 1921) there is heightened gas production. The increased extensibility, resulting from plasticization of the starch and softening of the gluten, and the heightened gas production coordinate to rapidly distend existing gas cells and produce numerous new ones in the matrix. Simultaneous lipid changes favor gluten-starch adherence and consequentially facilitate gas retention thereafter. The interplay of these factors results in the gross effect of "oven spring." Further rise in temperature slows and finally inactivates zymase at about 65°C (Frey and Landis, 1932), but coagulation of the gluten is probably well under way (Alsberg, 1928). The still higher temperature rise, besides releasing dissolved gases, effects vaporization of alcohol, organic acids, and water, and so replaces fermentation to a degree. Terminally, the coagulation and gelatinization become complete and permanently fix the structures.

Correlation of microscopic structures and crumb qualities: Three characteristics of the crumb are directly correlated with the microscopic findings of bread. Obviously, the coarseness of the grain is a function of the size of the gas cells: the finer the grain the smaller the gas cells. The texture basically corresponds to the average thinness of the cell walls, thin walls being "soft and silky" while thick ones feel rough and harsh. The color of the crumb is closely related to the intramural gas pockets. By analogy, other gas suspensions (e.g. whipped cream, whipped egg whites) become whiter as the gas bubbles become smaller and more numerous, so, in bread, the larger cells are too coarse to do this, but their walls become whiter with the appearance of numerous microscopic bubbles within them (as in Fig. 32). The grain, texture, and color of the crumb are also mutually interrelated by these intramural pockets which thin the walls by subdivision, lower the average diameter of the cells, and whiten the color. Thus, crumb quality may be accurately measured.

Summary

This paper makes reference to the lack of published information concerning the microscopic structure of breadstuffs, briefly reviews some of the previous work in the field, analytically discusses the problem of making suitable sections, and lists the highlights of the adopted microscopic procedure.

Thirty-six photomicrographs are presented and described as a series beginning with the ingredients, following with the progression of dough samples taken after each operation, and terminating with the baked bread. Three routine magnifications were used in order to include as many of the changes as possible. The descriptions concern the starch, yeast, gluten, fatty substances, and the gas cells produced by leavening.

The photomicrographs are discussed under six subdivisions:

1. *Mixing* results in an entirely homogeneous suspension of starch, yeast, and fats in a glutenous matrix. Flour particles lose their autonomy.

2. *Processing* improves the bread by grouping the starch and more efficiently sealing the gas pockets by gluten segregation. Molding and proofing are fundamental operations.

3. *Baking* changes, involving oven spring, starch gelatinization, coalescence of the lipids, and leavening are visually demonstrated in the photomicrographs.

4. *Shortening* distribution in bread dough is indicated by the technique used. The observations suggest a concentration of the shortening upon the starch-gluten interfaces, effected principally by mixing, and coalescing during baking into sizable collections of specifically stained material.

5. *Leavening* involves a preliminary diffusion away from the point of CO₂ formation. The heat of baking affects gas solubility, vaporization, enzymatic behavior, and gluten properties.

6. *The crumb qualities* (grain, texture, and to some extent color) are directly correlated with microscopic structures, particularly the small intramural gas cells.

Acknowledgments

The authors acknowledge the assistance of Dr. William F. Petersen, College of Medicine, University of Illinois, Chicago, and the H. Piper Baking Company, Chicago, in supplying facilities, samples, and cooperation.

Literature Cited

Alsberg, C. L.

1928 The role of starch in bread making. A chapter in "A comprehensive survey of starch chemistry" (R. P. Walton, editor). The Chemical Catalog Co., New York.

- Baker, J. C.
1941 The structure of the gas cell in bread dough. *Cereal Chem.* **18**: 34-41
— and Mize, M. D.
1941 The origin of the gas cell in bread dough. *Cereal Chem.* **18**: 19-34.
- Butterworth, S. W., and Colbeck, W. J.
1938 Some photomicrographic studies of dough and bread structure. *Cereal Chem.* **15**: 475-488.
- Frey, C. N., and Landis, Q.
1932 Colloidal aspects of baking chemistry. A chapter in "Colloid chemistry" (Jerome Alexander, editor), Vol. IV. The Chemical Catalog Co., New York.
- Herter, W.
1920 Die Unterscheidung der Weizen und Rog enstarke auf Grund ihrer Verkleisterungs-temperatur. *Textilberichte uber Wissenschaft, Industrie, und Handel, Mellands, Mannheim, Jahrg. 1. Abs. Chemie et Industrie* **5**: 78.
- Katz, J. R.
1915 Das Althackenwerden der Brotkrume vom physiologisch-chemischen Standpunkte betrachtet. *Z. physiol. Chem.* **95**: 104-126, 136-151. (Also in "A comprehensive survey of starch chemistry," R. P. Walton, ed., The Chemical Catalog Co., New York, pp. 100-117, also in *Baker's Weekly*, 1934 and 1935.)
- Scheffer, W.
1916 Mikroskopische Dunnschnitte durch Gelacke Z ges Getreide-, Muhlen-, u Backereiw. **8**: 6-10.
- Stamberg, O. E.
1939 Starch as a factor in dough formation. *Cereal Chem.* **16**: 769-780.
- Sullivan, B.
1940 The function of the lipids in milling and baking. *Cereal Chem.* **17**: 6, 661-667.
- Verschaefelt, E., and van Teutem, F. E.
1915 Die Anderung der mikroskopischen Struktur des Brotes beim Althackenwerden. *Z. physiol. Chem.* **95**: 130-135.

THE TESTING OF WHEAT QUALITY BY RECORDING DOUGH MIXER CURVES OBTAINED FROM SIFTED WHEAT MEALS¹

JOHN A. JOHNSON, JR., and C. O. SWANSON

Kansas Agricultural Experiment Station, Manhattan, Kansas

(Received for publication July 12, 1941)

Cereal investigators have attempted to devise numerous "short-cut" methods for testing wheat quality. A method which does not require refined flour is of distinct advantage as it eliminates the process of milling. Furthermore, a method that would not require the amount of material usually needed in experimental milling would be desirable for evaluating new wheat varieties early in a plant-breeding program. A rapid, reliable test for inherent quality of wheat would also be valuable to grain buyers.

¹ Contribution No. 77, Department of Milling Industry.

Review of Short Methods

The wheat-meal-time-fermentation test has been studied by Saunders and Humphries (1928), Cutler and Worzella (1931), Pelshenke (1933), Bayfield (1935), Swanson (1937), Swanson and Dines (1939), and others. The test, although a rapid method, is limited in scope because of the many factors affecting the time or the interval between placing the dough ball in the water and the observance of disintegration. Methods of tempering and grinding the meal, because of their effect on granulation, were found to influence the time. Proteases, protease activators, and wheat germ shortened the time, while the addition of bran made it longer. Bayfield (1935) pointed out that heat treatment of samples influences the time test.

The application of viscometric technique to wheat meals has been investigated by Morgan (1924), Durham (1925), and Bayfield (1932). Morgan reduced the wheat to floury fineness by two or three reductions on an Arcade flour mill. The method of reduction employed by Durham consisted of several grindings with an Enterprise coffee mill and siftings over 34 wire and 10 XX flour cloth. The final overs of the 34 wire and 10 XX flour cloth were passed between smooth rolls and again sifted over the same sieves. The resulting meal was relatively free from bran. Bayfield employed a hammer type of mill for reducing the wheat to floury granulation.

Geddes and Aitken (1935) described a micro milling and baking technique which has proved valuable in the testing of small samples of grain. Only 100 g of wheat for milling and 25 g of flour for a single bake were required. This method of testing small samples has been adopted at the North Dakota Agricultural Experiment Station (Harris and Sanderson, 1939).

The use of recording dough mixers in the study of mixing behavior of flours is now familiar to cereal workers. Although the application of these recording mixers has been limited almost entirely to refined flours, they also have been used on wheat meals. Malloch (1938), using the Malloch recording dough mixer, found a significant correlation ($r = +.88$) between the breaking point of meal doughs and flour doughs. This relationship was deemed valuable in an approximate classification of wheat samples. Rowland Clark² (unpublished data) has been using the National Swanson-Working micro recording dough mixer on meals obtained by grinding wheat on a Labconco mill and sifting through a 10 XX flour cloth. The advantages of a short process of preparation in testing small samples are obvious. Since it was found

² The authors wish to acknowledge valuable suggestions received from Mr. Clark, Chief Chemist, Shellabarger Mills, Salina, Kan-as

that preliminary treatments of the wheat samples, such as methods of grinding and sifting as well as water absorption, influence curve characteristics, it was decided to study these factors.

Methods

Two wheat varieties, Tenmarq and Chiefkan, both of high protein content and producing curves of widely different characteristics, were chosen for the studies of the effect of grinding, tempering, and absorption upon the type of curves produced. Flours from these two varieties were milled on the 65-barrel Kansas State College mill while a Hobart mill was employed to grind small portions of these wheat meals for use in the recording dough mixer.

The method of regulating the fineness of grinding with the Hobart mill was as follows: The burr gauge was placed on the 1½ mark and the center setscrew tightened until the burrs touched slightly when the grinder was in motion. This was considered as the initial standard setting. Moving the burr gauge to the No. 1 position gave the finest setting used, while moving to No. 2 gave the coarsest. The ground meal was sifted with a small rotary sifter, using a stack of sieves as in the bolting of flour. The amount of sifting for each operation and sieve system was measured by means of a time switch attached to the rotary sifter. Percentage of extraction was calculated on the basis of the clean air-dry wheat.

The curves were made by the National Swanson-Working micro recording dough mixer, using No. 9 spring setting. Both the flours and meals were weighed out to give 35 g of material on the 15% moisture basis. The amount of water required for optimum consistency for flours as well as meals was read from a graph in which protein content was plotted against absorption. This graph was constructed as follows: The proper absorption for both the low (near 9.5%) protein and the high protein content samples of each variety was carefully determined by the manual method. The two absorption values were plotted as ordinates and their respective protein contents as abscissas and a straight line drawn between the points thus established. The position of the protein figure on this line was then used as an indication of the proper absorption for the corresponding sample. Samples below 9.5% protein were treated as if they contained 9.5% protein. This was done since the linear relation between protein content and absorption does not seem to continue below 9.5% protein. Markley, Bailey, and Harrington (1937) have demonstrated the linear relationship between protein content and absorption. Karl F. Finney³ (unpublished data) uses such a method for estimation of absorption in the baking test.

³ Baking Technologist, Hard Winter Wheat Quality Laboratory, Manhattan, Kansas.

Experimental

In Figure 1 are shown the effects of absorption, grinding, and tempering upon the characteristics of the dough mixer curves. Curves 1, 2, 3, 4, 5, and 6, were prepared from Tenmarq and Chiefkan flours, as

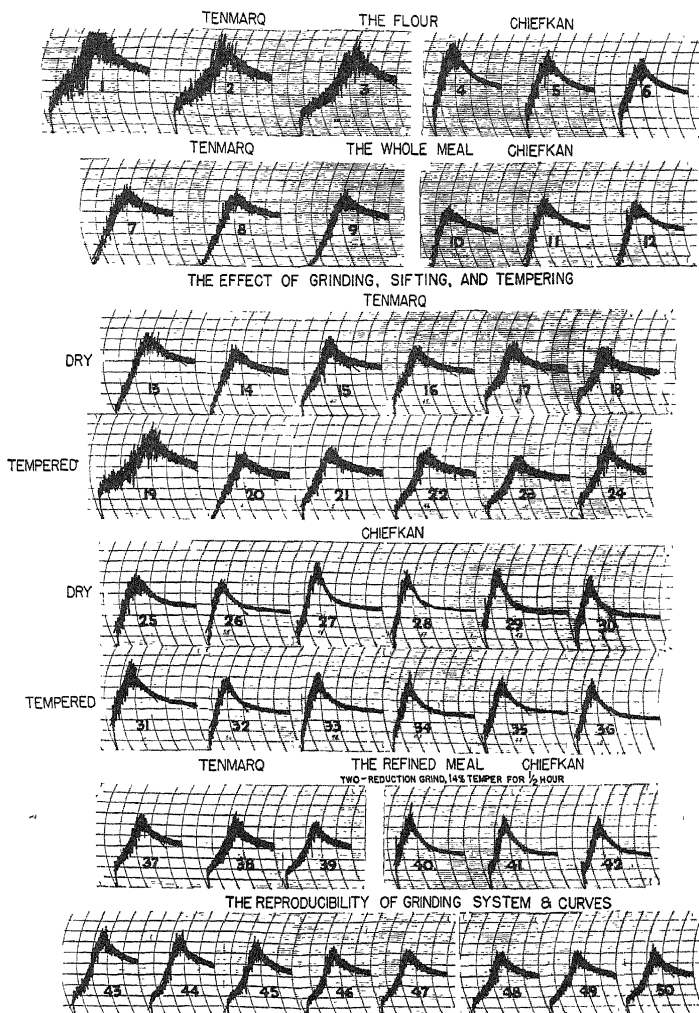


Fig. 1. Effects of grinding, sifting, and tempering on mixing-curve characteristics.

indicated in the figure, arranged in order of increasing absorption at 2% intervals starting at 63%. Directly below these curves are shown the corresponding whole-wheat-meal curves, ground only once through the Hobart mill with the finest setting. It is apparent that the whole-wheat-meal curves did not give quite as large differentiation between varieties as the flour curves. The meals were less sensitive to the amount of absorption and the differences in the time of development between the Tenmarq and Chiefkan were less than for the corresponding flours.

The effects of tempering, grinding, and sifting the wheats are shown in curves 13 to 36. Curves 13 to 18 are from Tenmarq wheat ground air-dry on the Hobart mill, sifted, and arranged in order of increasing fineness. Curve 19 was made for comparison from the Tenmarq flour using an optimum absorption. Curves 20 to 24 correspond to curves 14 to 18 with the exception that a 14% temper for one-half hour was used before grinding. Curves 18 and 24, obtained by a two-reduction grinding which resulted in the finest meal, approached No. 19 more closely. Tempering had comparatively little effect upon the character of a curve except a slight tendency to increase the time of development. The Chiefkan curves, 25 to 36, were obtained similarly to those of Tenmarq. No. 31 being made from the flour. These show, in comparison with the Tenmarq curves, 13 to 24, nearly as great a differentiation between these two varieties as is shown by the flour curves 19 and 31, respectively.

Curves 37 to 42 are from sifted meals of Tenmarq and Chiefkan, respectively, prepared by a two-reduction grinding with a 14% temper for 30 minutes and arranged in order of increasing absorption of 2% intervals starting at 70%. While increased absorption caused a slightly longer time of development and decrease in the height of curve, the differentiations between varieties shown by curves 37 to 39 (Tenmarq) and 40 to 42 (Chiefkan) are practically as great as shown between 1 to 3 and 4 to 6, made from the corresponding flours.

The reproducibility of curves from a two-reduction grinding and bolting system is shown in curves 43 to 47, which are arranged in order of grinding. Curve 43 was made from meal obtained on a cold mill. With continued grinding the mill becomes warmer and the effect is shown in the character of the curves being regularly reduced in curve height. This indicates that the curves from a meal ground on a cold mill will not be the same as when ground on a warm mill. For this reason a large sample of grain should be ground before grinding the test samples. Curves 48 to 50 were made from meal ground as one large sample and show the uniformity in curves when there is no variation in grinding.

Effects of Tempering

The effects of tempering are shown in Figure 2 and the corresponding data are given in Table I. The wheat samples were tempered to 11%, 12%, 13%, and 14% for 15, 30, and 60 minutes. In Table I the column headings under "Data from curves" are explained as follows:

TABLE I

THE EFFECT OF TEMPERING WHEAT UPON THE RESULTANT DOUGH MIXER CURVES

Variety	Curve No (Fig 2)	Treatment		Grind extrac- tion	Analytical data			Data from curves		
		Time	Temper		Mois- ture	Ash	Absorp- tion	Time of devel- opment	Curve height	Angle of slope
		<i>min</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>%</i>	<i>min</i>	<i>units</i>	<i>deg</i>
Tenmarq	1	0	0	38.0	9.5	0.87	67.1	2.4	7.1	74
"	2	15	11	48.0	10.3	0.96	68.7	2.5	6.8	75
"	3	15	12	47.3	10.7	1.01	69.4	2.6	7.0	71
"	4	15	13	41.3	12.0	1.02	71.9	2.6	7.1	80
Chiefkan	5	0	0	48.0	9.4	0.90	64.3	1.5	6.5	63
"	6	15	11	48.0	10.5	0.95	66.2	1.6	6.6	58
"	7	15	12	50.1	11.0	0.97	67.1	1.6	6.5	63
"	8	15	13	50.0	11.6	0.98	68.2	1.6	6.1	66
Tenmarq	9	30	11	52.7	11.0	0.97	69.8	2.6	6.8	81
"	10	30	12	51.3	11.2	0.99	70.4	2.5	6.5	81
"	11	30	13	50.0	11.6	0.99	71.1	2.7	6.1	87
"	12	30	14	50.7	13.0	1.02	73.9	2.9	6.6	88
Chiefkan	13	30	11	50.0	10.3	0.95	65.8	1.4	6.3	61
"	14	30	12	50.0	10.9	0.97	66.9	1.4	6.6	56
"	15	30	13	47.3	11.8	1.01	68.6	1.6	6.4	65
"	16	30	14	51.3	12.1	1.03	69.2	1.5	6.8	61
Tenmarq	17	60	11	44.7	10.6	0.93	69.7	2.6	6.2	78
"	18	60	12	49.3	11.0	0.92	70.5	2.6	6.2	84
"	19	60	13	50.7	12.0	0.94	72.4	2.7	6.7	74
"	20	60	14	57.3	13.1	1.03	74.6	3.1	6.8	79
Chiefkan	21	60	11	47.3	10.1	0.95	65.5	1.5	6.9	50
"	22	60	12	46.3	10.4	0.97	66.0	1.4	6.5	55
"	23	60	13	52.0	11.1	0.99	67.3	1.6	6.7	56
"	24	60	14	49.3	13.4	1.04	71.8	1.9	6.7	57

Time of development is determined by the number of curved vertical lines upon the chart paper passed before the dough reaches its maximum resistance to the revolving pins. The time to pass between two curved lines is about one minute. Height is given in the number of horizontal lines on the chart paper that are passed when the top is reached. Angle of slope is the number of degrees obtained by measuring the angle between lines drawn in the center of the upslope and the first part of the downslope.

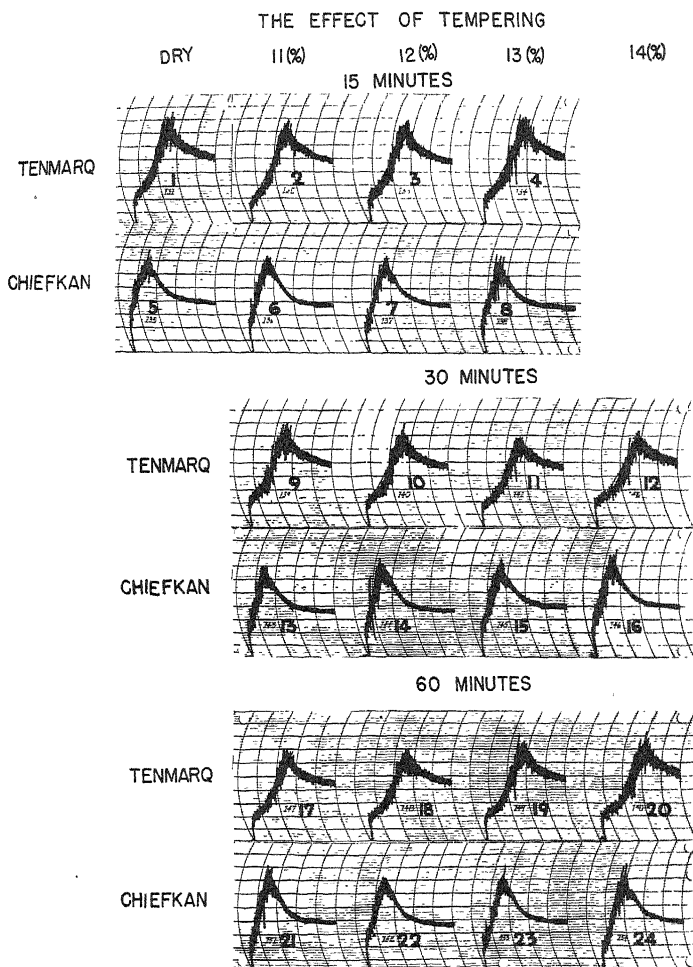


Fig 2. Effect of tempering on mixing curve characteristics.

The curves in Figure 2 indicate that the percent and length of temper had but little effect on the curve characteristics. Data in Table I also indicate that the length of temper had very little effect, but the amount of tempering water increased the moisture and ash contents and tended to increase the percentage of extraction as well as the absorption slightly.

With increased amount of tempering water and consequent larger moisture content, the time of development tended to be lengthened, but the heights of the curves and angles of the slopes indicate no definite trend. It appears that any tempering treatment, within the range studied, would aid in grinding without materially affecting the curve characteristics. It is very likely that a more refined meal could be obtained if the wheat were allowed to be tempered for a longer period, such as is customary in roller milling, but this would be impractical in this type of testing.

The system finally adopted in preparation of the sifted meals is as follows: The whole cleaned wheat, tempered to 14% moisture for 30

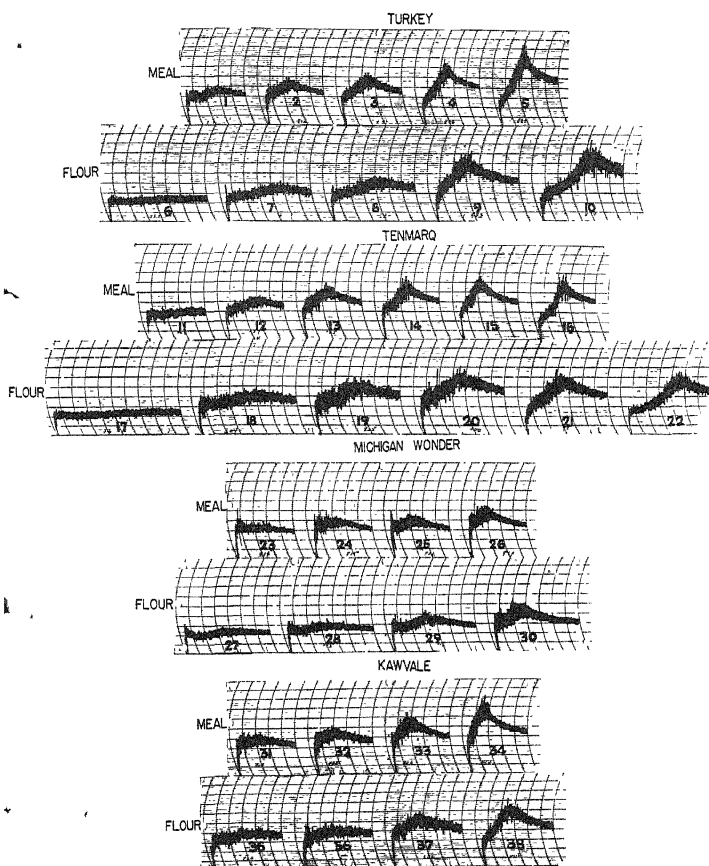


Fig. 3. Comparisons of meal and flour curves.

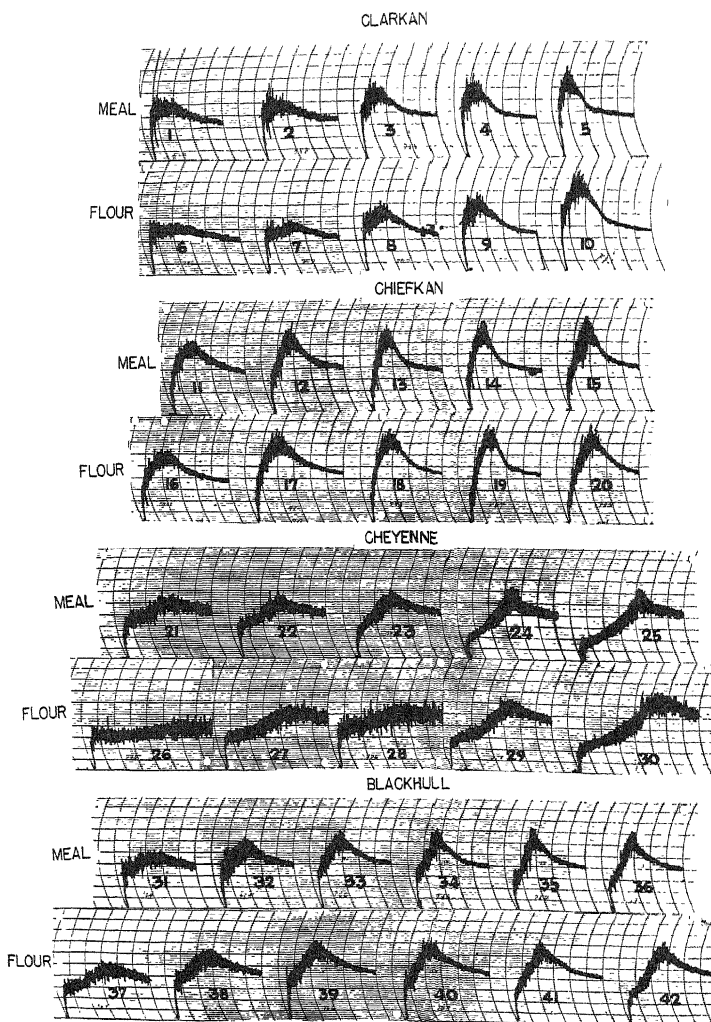


Fig 4. Comparisons of flour and meal curves

minutes, is ground, using the No. 2 setting on the Hobart mill. The ground meal is then sifted for four minutes, using the following stack of sieves: 26 wire, 50 GG, 70 GG, and 10 XX. The overs of the 26 wire are discarded. The overs of 50 GG and 70 GG are reground separately,

using the No. 1 setting, and again sifted over the 50 GG, 70 GG, and 10 XX for four minutes, the overs of the 50 GG and 70 GG being discarded. The overs of the 10 XX are reground, using the No. 1 setting and resifted on 10 XX flour cloth, the overs being discarded. The throughs of the 10 XX make up the meal used for making the curves. These meals are not free from bran specks, as might be expected, but the fineness is quite uniform.

Comparison of Sifted Meals with the Corresponding Flours of Several Varieties

Further studies were continued in comparing the curves made from meals and the corresponding flours. Several varieties of wheat were available, each variety consisting of a series of samples varying in protein content. The flours had been milled on a laboratory Allis-Chalmers mill. The meals were prepared by the method given in the preceding paragraph. The curves obtained on these meals and flours are shown in Figures 3 and 4 and analytical as well as other data are given in Table II. The meal and flour curves for each variety are arranged in order of increasing protein content. The absorption used for each meal and flour respectively was interpolated from a protein-vs.-absorption graph as explained previously.

The data in Table II show that the meals had higher ash, higher protein, and higher absorptions but lower moisture percentages than the flours. It seems likely that the higher absorption of the meals was due partly to their higher protein content as compared with that of the flours.

The curves in Figures 3 and 4 indicate that the meal curves bring out characteristics of the wheat varieties in a way similar to the flour curves. Although the meals are slightly higher in protein content, and, as will be shown later, protein content influences certain characteristics in the curves, yet the curve characteristics of the various varieties are different for both the flour and the meal.

Correlation Coefficients

In order to test how closely the characteristics of the curves obtained from the sifted wheat meals agree with those from the flours and also to test the relation of protein content to these characteristics, the correlation coefficients were calculated and tabulated in Table III. The figures for the calculation of these coefficients were obtained from the data on the curves, meals, and flours given in Table II. Data on curves from the meals and flours of the lowest protein content were not included in the statistical study because of the uncertainty in locating suitable points of measurements.

The correlation coefficients as shown in Table III indicate the following: Protein content of the meal or flour is positively correlated with the height of curves at optimum consistency, while it is negatively correlated with the angle of slope. The protein content of the meal is posi-

TABLE II
A COMPARISON OF SIFTED MEAL WITH THE CORRESPONDING FLOUR
OF SEVERAL VARIETIES

Variety	Curve No. (Fig. 3)	Material	Grind extraction	Analytical data				Data from curves		
				Moisture	Ash	Protein	Absorption	Time of development	Curve height	Angle of slope
			%	%	%	%	%	min	units	deg
Turkey	1	Meal	46.8	12.8	0.99	9.6	57.9	—	—	—
"	2	"	48.8	12.5	1.00	10.5	59.8	2.5	4.3	138
"	3	"	48.0	12.7	1.06	11.9	63.1	2.5	4.7	133
"	4	"	49.2	12.1	1.07	15.5	69.7	2.4	5.7	99
"	5	"	48.0	11.9	1.15	17.4	72.7	2.3	7.0	72
"	6	Flour	—	14.3	0.38	8.2	57.2	—	—	—
"	7	"	—	14.2	0.39	9.3	57.0	4.6	3.5	162
"	8	"	—	14.0	0.40	10.2	58.1	3.9	4.2	156
"	9	"	—	13.7	0.44	11.8	59.6	3.0	6.0	115
"	10	"	—	13.9	0.45	15.5	66.8	4.4	6.7	118
Tenmaŕq	11	Meal	53.6	12.6	1.03	9.0	57.1	—	—	—
"	12	"	46.8	12.5	1.03	10.3	58.7	3.2	4.1	149
"	13	"	47.2	12.3	1.08	12.3	62.9	2.4	5.1	125
"	14	"	46.0	12.2	1.07	13.6	65.1	2.5	5.4	115
"	15	"	48.0	12.2	1.10	14.6	66.9	2.1	5.8	106
"	16	"	46.4	12.4	1.16	17.6	73.6	2.5	5.6	96
"	17	Flour	—	14.7	0.40	7.9	57.0	—	—	—
"	18	"	—	14.3	0.39	9.3	56.2	5.2	4.0	165
"	19	"	—	14.3	0.37	10.8	58.2	4.3	5.1	145
"	20	"	—	14.2	0.38	11.8	60.0	3.5	5.8	137
"	21	"	—	14.1	0.37	13.3	62.8	3.4	5.5	128
"	22	"	—	14.0	0.43	15.3	66.0	4.8	5.6	132
Michigan Wonder	23	Meal	46.8	11.8	0.89	8.9	52.3	—	—	—
"	24	"	46.4	12.2	0.76	9.6	53.0	2.4	3.8	162
"	25	"	49.5	12.2	0.77	10.2	55.4	2.4	3.9	157
"	26	"	56.0	11.9	0.75	13.0	59.7	2.0	4.8	132
"	27	Flour	—	14.3	0.37	8.3	53.2	—	—	—
"	28	"	—	14.2	0.37	8.7	53.1	3.6	2.8	168
"	29	"	—	14.4	0.37	9.0	53.3	3.1	3.8	150
"	30	"	—	14.4	0.34	10.8	55.9	2.8	4.3	138
Kawvale	31	Meal	54.0	12.8	0.99	9.6	55.5	2.4	3.9	158
"	32	"	50.0	12.6	1.10	10.2	56.6	1.9	4.5	143
"	33	"	50.0	12.5	1.00	11.9	59.8	1.9	5.4	113
"	34	"	48.8	12.5	0.96	14.9	65.9	1.6	6.7	81
"	35	Flour	—	13.6	0.40	8.3	53.5	3.2	3.9	165
"	36	"	—	13.7	0.40	8.9	53.6	3.0	4.0	160
"	37	"	—	13.9	0.38	10.4	55.5	2.7	5.2	142
"	38	"	—	14.1	0.38	13.3	61.3	2.5	6.1	110

TABLE II.—Continued

Variety	Curve No. (Fig. 4)	Material	Grind extraction	Analytical data				Data from curves		
				Moisture	Ash	Protein	Absorption	Time of development	Curve height	Angle of slope
Clarkan	1	Meal	$\frac{w}{\%}$	$\frac{w}{\%}$	$\frac{w}{\%}$	$\frac{w}{\%}$	$\frac{w}{\%}$	min	units	deg
"	2	"	51.2	12.4	0.89	9.4	53.3	2.0	4.8	153
"	3	"	53.2	12.4	0.74	9.5	53.3	1.7	4.7	137
"	4	"	52.8	12.4	0.67	11.0	55.7	1.3	5.6	108
"	5	"	51.2	12.4	0.65	12.2	58.2	1.3	5.6	103
"	6	"	57.2	12.2	0.81	14.1	60.6	1.0	6.3	77
"	7	Flour	—	14.4	0.32	8.3	52.8	2.5	4.0	167
"	8	"	—	14.3	0.31	8.7	52.7	2.3	4.2	148
"	9	"	—	14.7	0.30	9.9	54.0	1.8	5.3	121
"	10	"	—	14.3	0.32	11.3	55.8	1.6	5.8	107
"	11	"	—	14.4	0.35	12.8	59.4	1.4	7.3	82
Chiefkan	11	Meal	44.8	12.9	1.14	11.8	60.0	1.8	5.8	102
"	12	"	44.4	12.9	1.04	13.0	62.5	1.7	6.5	79
"	13	"	45.2	12.6	1.12	14.1	64.4	1.6	6.5	59
"	14	"	46.8	12.2	1.12	15.3	66.5	1.4	6.8	55
"	15	"	45.2	12.2	1.17	15.8	67.6	1.7	7.1	60
"	16	Flour	—	14.6	0.41	9.3	55.8	2.3	5.9	105
"	17	"	—	14.3	0.37	10.7	57.8	1.9	7.1	96
"	18	"	—	14.6	0.37	11.6	59.7	1.7	7.3	76
"	19	"	—	14.4	0.39	13.1	62.3	1.8	7.5	61
"	20	"	—	14.2	0.43	14.6	64.5	1.9	7.4	78
Cheyenne	21	Meal	45.0	12.2	0.98	10.6	57.3	—	—	—
"	22	"	43.2	12.6	1.04	12.5	62.4	3.3	4.9	138
"	23	"	48.0	12.2	1.04	12.9	62.6	3.1	5.3	135
"	24	"	46.4	12.6	1.17	15.3	69.2	3.5	5.4	113
"	25	"	49.2	12.5	1.15	16.7	72.0	4.4	5.3	125
"	26	Flour	—	14.3	0.37	9.2	55.8	—	—	—
"	27	"	—	14.3	0.37	10.4	57.2	4.8	5.2	148
"	28	"	—	14.2	0.40	11.2	59.0	5.0	5.2	159
"	29	"	—	13.7	0.44	13.2	61.0	4.5	5.7	134
"	30	"	—	14.5	0.44	14.8	65.5	5.5	6.6	130
Blackhull	31	Meal	54.4	12.6	1.09	9.4	55.1	2.3	4.7	141
"	32	"	53.2	12.4	0.98	10.3	56.2	2.1	5.4	109
"	33	"	52.2	11.8	0.86	11.9	59.0	1.9	6.0	99
"	34	"	52.8	12.4	0.91	13.2	63.5	1.8	6.2	88
"	35	"	52.0	12.2	0.90	14.5	66.0	1.7	6.3	79
"	36	"	52.0	11.5	0.92	16.6	69.5	1.7	6.3	80
"	37	Flour	—	14.6	0.35	8.4	55.3	3.3	4.5	143
"	38	"	—	14.4	0.35	9.1	54.9	2.8	5.7	128
"	39	"	—	14.2	0.35	10.5	56.5	2.3	6.4	106
"	40	"	—	14.2	0.36	11.8	59.0	2.3	6.5	99
"	41	"	—	14.3	0.38	13.1	61.7	2.2	6.4	95
"	42	"	—	14.1	0.41	15.1	65.2	2.5	6.5	101

tively correlated with that of the flour. In this study no significant correlation was demonstrated between protein content and the time of development as measured to the peak of the curve. At low protein

TABLE III
CORRELATION COEFFICIENTS COMPUTED FROM ANALYTICAL AND
CURVE DATA
N = 35

Variables correlated		Correlation coefficient
X	Y	<i>r_{xy}</i>
Meal, protein (%)	Flour, protein (%)	+ .9623¹
Meal, protein (%)	Meal, time of development (<i>min</i>)	+ .1324
Flour, protein (%)	Flour, time of development (<i>min</i>)	+ .1070
Meal, protein (%)	Meal, height of curve (<i>units</i>)	+ .7207
Flour, protein (%)	Flour, height of curve (<i>units</i>)	+ .6863
Meal, protein (%)	Meal, angle of slope (<i>deg</i>)	- .7097
Flour, protein (%)	Flour, angle of slope (<i>deg</i>)	- .5841
Meal, angle of slope ² (<i>deg</i>)	Flour, angle of slope ² (<i>deg</i>)	+ .9221
Meal, time of development (<i>min</i>)	Flour, time of development (<i>min</i>)	+ .8738
Meal, height (<i>units</i>)	Flour, height (<i>units</i>)	+ .9159

¹ Bold type correlations are highly significant, since with 1% level $r_{xy} = 0.425$.

² Angle of up-and-down slopes

levels the time of development might appear to be longer than at higher protein levels. However, as it was impossible with the lowest protein samples to determine this peak from the curve accurately, these samples were not used in calculating the correlation coefficients. While the time of development as shown in Table II indicates a trend to decrease with increasing protein in all varieties except Cheyenne, this trend is not uniform and therefore the correlation coefficient between these variables is not significant. Of most interest is the existence of a high positive correlation between curve measurements of the meal and of the flours in respect to time of development, height of curve, and angle of the up and down slope. While these meal and flour curve characteristics are highly correlated, they are not identical, but the differences between them vary together. The meal curves in comparison with the flour curves tend to have a shorter time of development, a smaller up and down slope angle, and about the same height. It is apparent from Figures 3 and 4 that not all varieties tended to give curves in which the co-variance of meal and flour characteristics are the same. The meal curves of Clarkan, Chiefkan, and Blackhull show smaller differences in the time of development in comparison with the flour curves than do the other varieties. Tenmarq and Cheyenne show the greatest difference in this respect. The varietal factors must be considered when interpreting the meal curves.

Since protein content of the meal is highly correlated with protein content of the flour and since the protein content is positively correlated with the height of the curves and negatively correlated with the angle of slopes and also since the meal curves are correlated with the flour curves, it appears that the meal curves may be used as an approximate indication of the protein content, provided the time of development is taken into account. A classification of wheats into high, medium, and low protein

content can be made on the basis of curve characteristics. Furthermore, since the time of development varies more than any of the other characteristics among the varieties and also since this time is not correlated with the protein content, the time of curve development can be used to characterize the varieties in a general way. With the possible exception of Cheyenne the varieties of longer mixing times are generally considered to have a wider range of adaptability for bread-making.

Conclusions

A method has been described which makes it possible to obtain satisfactory curves on the recording dough mixer, using sifted wheat meals instead of flours. While the curves obtained from the meals are not exactly like those obtained from the flours, yet they are closely correlated in the characteristics of height, time of development, and the angles made by the up and down slopes of the curves. These correlations are statistically significant and hence the dough-mixer curve characteristics of a wheat may be observed almost as well from the meal curve as from the flour curve.

Literature Cited

- Bayfield, E. G.
 - 1932 A whole wheat meal viscosity test for detecting certain types of poor milling wheats. *Cereal Chem.* 9: 473-484.
 - 1935 Soft winter wheat studies. IV. Some factors producing variations in wholemeal "time" data. *Cereal Chem.* 12: 559-568.
- Cutler, G. J., and Worzella, W. N.
 - 1931 A modification of the Saunders' test for measuring "quality" of wheats for different purposes. *J. Am. Soc. Agron.* 23: 1000-1009.
- Durham, R. K.
 - 1925 Effect of hydrogen peroxide on relative viscosity measurements of wheat and flour suspensions. *Cereal Chem.* 2: 297-305.
- Geddes, W. F., and Aitken, T. R.
 - 1935 An experimental milling and baking technique requiring 100 grams wheat. *Cereal Chem.* 12: 696-707.
- Harris, R. H., and Sanderson, T.
 - 1939 A comparison between Allis-Chalmers and micro milling techniques on North Dakota hard red spring wheats. *Cereal Chem.* 16: 619-625.
- Malloch, J. G.
 - 1938 Some results with a new recording mixer for use with small samples. *Cereal Chem.* 15: 423-438.
- Markley, M. C., Bailey, C. H., and Harrington, F. L.
 - 1939 The colloidal behavior of flour doughs. VI. Dough formation from flours of diverse types. *Cereal Chem.* 16: 271-279.
- Morgan, R. W.
 - 1924 A practical application of the viscosimeter to the mill. *Cereal Chem.* 1: 288-292.
- Pelshenke, P.
 - 1933 A short method for the determination of gluten quality of wheat. *Cereal Chem.* 10: 90-96.
- Saunders, H. H., and Humphries, S.
 - 1928 The Saunders test. *J. Natl. Int. Agr. Bot.* 2: 34.
- Swanson, C. O.
 - 1937 Factors which influence results in the wheat-meal-time-fermentation test. *Cereal Chem.* 14: 419-433.
- and Dines, F. T.
 - 1939 The wheat-meal-time-fermentation test. II. Effect of proteases, protease activators, and protease inhibitors. *Cereal Chem.* 16: 168-177.

THE FERMENTOMETER, A GAS-PRODUCTION MEASURING DEVICE

ELMER F. GLABE

Stein, Hall Mfg. Co., Chicago

(Received for publication May 13, 1941)

Various types of pressure recording instruments for measuring gas production in doughs have been built. Sandstedt and Blish (1934) proposed the use of a "pressuremeter." Bailey (1939) suggested an apparatus which indicated both gas production and gas losses in fermenting doughs. Eisenberg (1940b) suggested another type of volumetric gas measuring apparatus. Other methods for volumetric measurement of this factor have been described by Brabender (1933) and Elion (1933). Eisenberg (1940a) doubts the contention of Sandstedt and Blish (1936) that gas production under pressure proceeds at the same rate as at atmospheric pressure.

Materials and Methods

A manometric apparatus was constructed embodying the principles used by Sandstedt and Blish in their pressuremeter and by Bailey in his gas-production and gas-retention apparatus (Fig. 1). It consists of a mercury manometer and a meter stick mounted on a brass plate, which has a rubber gasket the size of the top of a pint Mason jar countersunk into its underside. The fermentation chamber is the pint-size special-glass jar. This rests in a stand made of a brass plate with threaded rods mounted vertically in each corner. The apparatus has the advantage of using the idea of Bailey for the determination of the gas retention factor as well as the total gas production factor. This can be accomplished by using measured quantities of solutions of sodium chloride for total gas produced, or sodium hydroxide for gas retained. The dough is put into a screen-wire receptacle which in turn rests on a heavy copper wire base which keeps the wire basket above the solution.

In operation, the jar containing the dough and the proper solution are placed into the stand with the manometer set down over it and carefully adjusted so that the top of the jar and the rubber gasket meet exactly. These are then brought together tightly by means of wing nuts turning on the threaded rods set in each corner of the brass plates.

Doughs are made from 200 g of flour containing 15% moisture (or its equivalent in dry weight), 120 ml water, and 6 g of yeast. Other ingredients are added by dry mixing with the flour. Ten percent aliquot fractions of dough are weighed off carefully and placed into the dough baskets. All fermentation tests are carried out at a constant

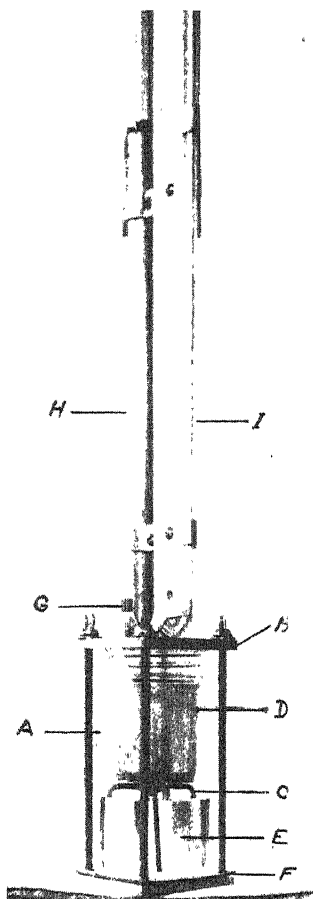


Fig. 1. The fermentometer *A*, pint-size glass jar, *B*, brass plate 3" x 3" with rubber gasket countersunk in underside; *C*, heavy copper wire stand, *D*, screen wire (40-mesh) dough basket, *E*, solution—23% NaCl or 23% NaOH, *F*, rubber gasket; *G*, automobile tire valve for releasing pressure; *H*, brass pipe $\frac{1}{4}$ "; *I*, mercury-filled glass manometer.

temperature of 30°C by means of a water bath. Mixing, dough scaling, and mounting operations take about 6 minutes per dough. About 9 minutes are allowed for the apparatus and the dough piece to come to temperature. Exactly 15 minutes after the flour and the yeast water are first mixed, the mercury in the manometer is adjusted to 0 by venting the gas in the fermentation chamber by means of a valve in the top plate of the apparatus.

In this laboratory a set of eight units is operated simultaneously in a specially constructed water bath. The latter is a stainless steel box $12'' \times 14'' \times 10''$ deep. It is fitted with a thermo-regulator, knife-blade heater, cooling coils, and mechanical stirring apparatus. In the water bath the units rest on racks which raise them an inch above the floor of the bath, permitting thorough circulation of the water (Fig. 2).

Readings in millimeters of pressure are taken hourly. The results are calculated by means of Boyle's law to the amount of carbon dioxide

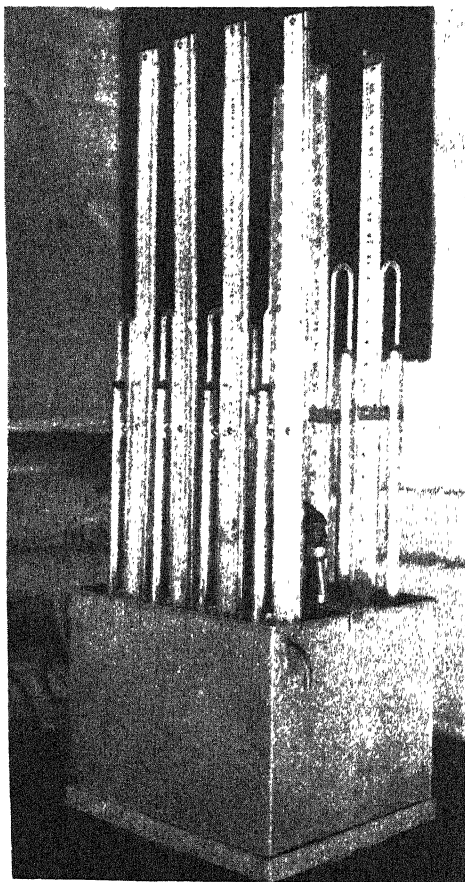


Fig. 2. Eight fermentometer units in a stainless steel constant-temperature bath.

gas evolved by 100 g of flour at 15% moisture at 760 mm pressure and 30°C.

Calibration of the Apparatus

A series of seven doughs were made in the manner described in the foregoing paragraphs, using a well blended flour and a standard yeast. Duplicate aliquots were taken from each dough and gas production determined. Readings were taken at each half hour for five hours. The composite results of these 14 determinations are given in Table I.

TABLE I
GAS PRODUCTION VALUES

Time	Average, 14 determinations	Variation, 14 determinations
<i>hrs</i>	<i>ml</i>	<i>ml</i>
1½	179	±14.0
1	341	±17.0
1½	615	±31.0
2	907	±40.0
2½	1170	±25.0
3	1314	±13.5
3½	1399	± 7.5
4	1466	± 8.5
4½	1520	± 8.0
5	1568	±17.0

From Table I it will be noted that variation is greatest at the 1½, 2, and 2½ hour readings. The data also bring out the fact that variation is least at the period of final readings (4 and 4½ hours). The 4½-hour period has been chosen by this laboratory as the point for total gas production readings since at this point the degree of accuracy of this apparatus is greatest.

Effect of Varying Pressure on Gas Production

The following experiments were made on four doughs with duplicate portions from each, making a total of eight determinations for each experiment. Readings were taken hourly to 4 hours and again at 4½ hours, under conditions, respectively, as follows: (1) gas pressure allowed to build up for the full 4½ hours; (2) gas pressure released at the end of each one-hour period, apparatus reset to zero; (3) tests started at pressure of 100 mm by pumping air into the units after sealing, pressure allowed to build up for the full period of 4½ hours; and (4) the same as (3), except that the pressure was reset each hour to the starting point of 100 mm. The results in ml per 100 g flour at 15% moisture are shown in Figure 3. It will be noted that the curve representing Experiment 1 shows the highest gas volumes throughout the test period. There was, however, no significant difference between

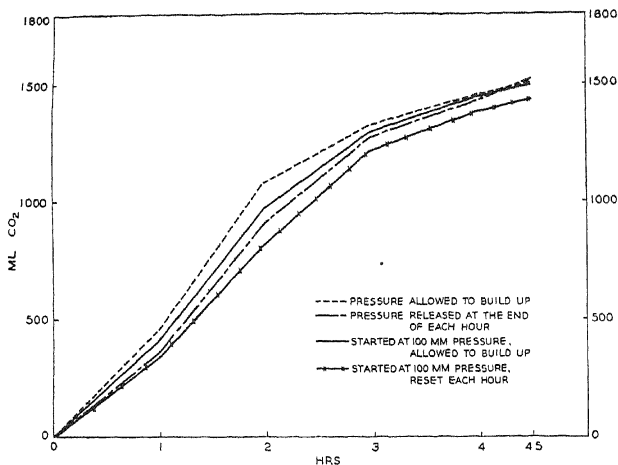


Fig. 3 Effect of varying pressure on gas production

the final readings of this experiment and those of Experiments 2 and 3. The greatest variation between experiments (260 ml) was at the second hour reading. Experiment 4 showed the only significant differences throughout the period of the test.

Summary

A new variation of the pressuremeter, the fermentometer, is described. This apparatus is designed to register gas production and gas retention during dough fermentation. Calibration and degree of accuracy data on gas production are given.

Curves indicating the effect of pressure on gas production in dough are presented.

Literature Cited

- Bailey, C. H.
1939 Measuring fermentation rate and gas losses in dough. *Cereal Chem.* **16**: 665-671.
- Brabender, C. W.
1933 Vier Jahre Farinograph und Fermentograph. *Mehlprobleme* **2**: 47-49.
- Eisenberg, S.
1940a Gas production in yeast fermentation and its applications. I. Volumetric vs. manometric methods for the determination of gas production. *Cereal Chem.* **17**: 417-429.
1940b Gas production in yeast fermentation and its applications. II. A volumetric method for the study of gas production. *Cereal Chem.* **17**: 430-447.
- Elion, E.
1933 A simple volumetric method for measuring gas production during dough Fermentation. *Cereal Chem.* **10**: 245-249.
- Sandstedt, R. M., and Blish, M. J.
1934 Yeast variability and its control in flour gassing power tests. *Cereal Chem.* **11**: 368-383.
1936 Effect of pressure on rate of gas production in yeast fermentation. *Cereal Chem.* **13**: 789-790.

BROMIDE RESIDUES IN CEREALS FUMIGATED WITH METHYL BROMIDE

L. S. ROEHM, S. A. SHRADER, and V. A. STENGER

The Dow Chemical Company, Midland, Michigan

(Received for publication July 18, 1941)

Since the excellent pioneer work of Mackie (1937, 1938), Hawkins (1938, 1939) and their collaborators, methyl bromide has been widely accepted for the fumigation of nursery stock and fresh or dried fruits and vegetables. In view of the economy, efficiency, and thoroughness of penetration of this fumigant, much interest has been aroused concerning its use in the cereal industry. However, the finding of somewhat higher bromide residues in certain products such as milled grains after fumigation on a laboratory scale (Stenger, Shrader, and Beshgetoor, 1939; Dudley *et al.*, 1940) has raised a question as to whether such residues might be objectionable in the fumigated products (Cotton, 1941). Until recently it had been feared that the residual bromide might consist in part of free methyl bromide, no method having been available for distinguishing between the various forms of bromide present in these small amounts.¹ Now a method for this purpose has been developed and the bromide remaining in fumigated flour has been shown to be largely inorganic immediately after fumigation and entirely inorganic within several days (Shrader, Beshgetoor, and Stenger, 1941). Thus any fear of methyl bromide remaining in the product has been dispelled.

It is the purpose of this paper to present additional information regarding the bromide residues in flour and other cereals, and to show that not only do the residues consist of inorganic bromides, but the magnitudes of these residues are lower under the recommended commercial fumigation conditions than had previously been expected from experiments conducted on a laboratory scale. The amounts of bromide retained by a fumigated product depend greatly upon the nature of the material and upon the conditions under which fumigation takes place, in particular upon the concentration of methyl bromide, ratio of methyl bromide to product, time of exposure, temperature, type of fumigation (vacuum or atmospheric), and relative humidity in the case of the atmospheric type. To duplicate commercial conditions in the laboratory is therefore difficult.

¹ Following the completion of the experimental work here reported, there has been published a paper by E. P. Laug (1941). Although Laug concludes correctly that only nonvolatile bromide remains after a sufficient time of airing, his method for determining volatile bromide is open to question. Furthermore, his laboratory-scale tests on cereals were made almost exclusively with such a high concentration of methyl bromide (about 16 lbs per 1,000 cu ft) and high ratio of fumigant to product (apparently on the order of 500 lbs CH_3Br to 1,000 lbs of product) that the results have scarcely any significance for commercial purposes.

TABLE I
BROMIDE CONTENT OF FUMIGATED WHEAT PRODUCTS

Product and package	CH ₃ Br per 1,000 cubic feet	Ap- prox. load	CH ₃ Br per 1,000 lbs load	Temp	Percent bromide							
					Control		Outer layer of bag		Center of bag		Composite	
					Total	Inorg.	Total	Inorg.	Total	Inorg.	Total	Inorg
White flour (100-lb cloth bags)	lbs	lbs	lbs	°F	%	%	%	%	%	%	%	%
	1.07	48,200	0.104	74-84	.0010	—	.0021	—	.0028	—	.0028	—
	1.28	48,200	0.125	76	.0009	.0009	.0031	.0033	.0023	.0023	.0025	.0027
Whole-wheat flour (2.5-lb paper bags, in cartons)	1.49	84,000	0.083	51-77	.0001	—	.0043	—	.0018	—	.0023	—
	1.07	48,200	0.104	74-84	.0003	—	—	—	—	—	.0028	—
	1.28	48,200	0.125	76	.0002	.0003	—	—	—	—	.0038	.0038
Whole-wheat flour, extra fine (100-lb cloth bags)	1.49	84,000	0.083	51-77	.0003	.0002	—	—	—	—	.0027	.0025
	1.49	84,000	0.083	51-77	.0002	—	.0044	—	.0022	—	.0022	.0024
	1.28	48,200	0.125	76	.0008	.0008	—	—	—	—	.0101	.0096
100% wheat germ (2-lb paper bags)	1.07	48,200	0.104	74-84	.0003	—	.0018	—	.0015	—	.0013	—
	1.49	84,000	0.083	51-77	.0002	—	.0061	—	.0040	—	.0047	—
	1.07	48,200	0.104	74-84	.0006	—	.0009	—	.0008	—	.0008	—
Whole grain (soft red winter wheat, 100-lb cloth bags)	1.49	84,000	0.083	51-77	.0002	—	.0012	—	.0006	—	.0009	.0009
	1.28	48,200	0.125	76	.0004	.0004	.0036	.0037	.0027	.0023	.0028	.0024
	1.49	84,000	0.083	51-77	.0003	—	.0045	—	.0075	—	.0050	—

In Table I are shown data obtained during commercial atmospheric fumigations of various wheat products. The vault employed had a capacity of 4,700 cubic feet and in each case was well loaded. The fumigation time was 24 hours and the insect kill was consistently 100%. (In a few other experiments not reported here these dosages were found insufficient to kill all insects at considerably lower temperatures.) The samples for analysis were placed in sealed containers, shipped to the laboratory, and analyzed after standing open for one week in the same containers. Analyses for total and inorganic bromides were made by the procedure of Shrader, Beshgetoor, and Stenger (1941). An experimental error of $\pm 0.0005\%$ bromide in any result is possible.

Since the amounts of bromide retained by white flour and whole-wheat flour have been reported as from 0.0042% to 0.0091% (Dudley *et al.*, 1940; Stenger, Shrader, and Beshgetoor, 1939; Cotton, 1941) on the basis of laboratory tests, it is evident from Table I that the retentions are generally lower following commercial fumigations. The reason for this difference may be illustrated as follows: In laboratory fumigations one usually treats small quantities of product, say a pound, in a drum with a capacity of perhaps 7 cubic feet. To obtain a dosage of one pound of methyl bromide per 1,000 cubic feet, 0.007 pound of fumigant would be needed, or a ratio of 7 pounds to 1,000 pounds of product. Absorption of part of this methyl bromide would not change its concentration appreciably. In contrast, the ratios employed in the commercial fumigations listed in Table I were on the order of 0.11 pound of methyl bromide or less to 1,000 pounds of product. Partial absorption of this lowers the concentration considerably,

TABLE II
RESIDUAL BROMIDE IN FUMIGATED CEREAL PRODUCTS

Prepared dry cereal breakfast-food products	Bromide before fumigation		Bromide after fumigation	
	Total	Inorganic	Total	Inorganic
	%	%	%	%
A Wheat	.0011	.0014	.0025	.0024
B Corn	.0011	.0015	.0020	.0028
C Bran	.0011	.0012	.0027	.0027
D Wheat	.0011	.0012	.0018	.0017
E Wheat	.0007	.0012	.0021	.0025
F Mixed grains	.0005	.0012	.0011	.0014
G Mixed grains	.0010	.0019	.0024	.0024
RAW CEREALS				
H Rolled oats	.0010	.0014	.0145	.0149
I Corn meal	.0022	.0017	.0072	.0064
J Farina	.0012	.0014	.0070	.0065

and since the final absorption is a function of the final concentration, one finds that smaller amounts of bromide are retained when smaller ratios of fumigant to product are employed.

Table I also shows that within the limits of experimental error the bromide residues are all inorganic except possibly for a small fraction in the case of wheat germ.

A number of prepared cereals were fumigated on a semicommercial scale to secure information on the amounts of bromide retained. Each sample was in its original restaurant-size or retail-size package and a full carton of the packages of each product was used in the test. All the cartons were fumigated simultaneously with 0.75 pound of methyl bromide for 18 hours in a 500-cubic-foot vault at 70°F. The dosage thus corresponded to 1.5 pounds per 1,000 cubic feet, or 4.05 pounds per 1,000 pounds of product (the load in the chamber was brought up by the addition of 135 pounds of shorts). The samples were analyzed after standing for one week in the original containers and the data obtained are shown in Table II. In only three cases were the retentions appreciably high, and full-scale commercial fumigation of these three would likely show lower retentions if a lower ratio of fumigant to product were used.

TABLE III
LABORATORY FUMIGATION OF WHOLE-WHEAT FLOUR

Hours aired	Total bromide	Inorganic bromide	Organic bromide
	%	%	%
0.5	.0299	.0178	.0121
4	.0244	.0183	.0061
24	.0213	.0197	.0016
48	.0210	.0199	.0011
96	.0202	.0196	.0006
168	.0199	.0197	.0002
Control, not fumigated	.0003	.0004	...

To show the states in which bromide occurs in fumigated wheat products at various times after fumigation, analyses were made of laboratory-fumigated flour, starch, and gluten. The conditions were purposely made conducive to the absorption of larger amounts of bromide in order to obtain greater variations. In Table III and Figure 1 are presented data found in the fumigation of whole-wheat flour with two pounds of methyl bromide per 1,000 cubic feet for 24 hours at 80°F. The dosage corresponds to a ratio of 330 pounds of methyl bromide to 1,000 pounds of product, since only 3 pounds of product were fumigated in a 500 cubic foot chamber.

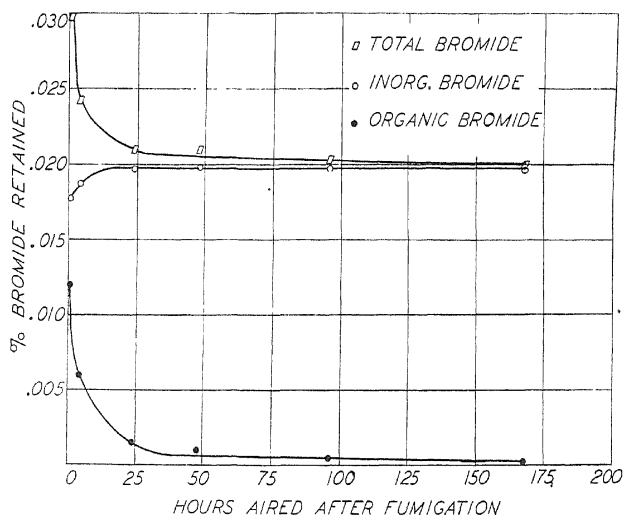


Fig 1. Retention of bromide by whole-wheat flour.

TABLE IV
LABORATORY FUMIGATION OF WHEAT STARCH

Hours aired	Total bromide	Inorganic bromide	Organic bromide
	%	%	%
0.5	.0076	.0032	.0044
4	.0034	.0032	.0002
24	.0031	.0031
48	.0031	.0031
96	.0031	.0030
168	.0032
Control, not fumigated	.0006	.0006

TABLE V
LABORATORY FUMIGATION OF GLUTEN

Hours aired	Total bromide	Inorganic bromide	Organic bromide
	%	%	%
0.5	.0334	.0292	.0042
4	.0311	.0286	.0025
24	.0312	.0288	.0024
48	.0306	.0283	.0023
96	.0306	.0287	.0019
168	.0303
Control, not fumigated	.0016	.0018

TABLE VI
LABORATORY FUMIGATIONS OF WHEAT PRODUCTS AT DIFFERENT HUMIDITIES

Product	Relative humidity during fumigation	Hours aired after fumigation	Total bromide retained
	%		g/g
White flour	30	1	.0354
	30	168	.0230
	70	1	.0146
	70	168	.0144
Whole-wheat flour	30	1	.0296
	30	168	.0199
	70	1	.0178
	70	168	.0160
Wheat starch	30	1	.0261
	30	168	.0004
	70	1	.0025
	70	168	.0018
Gluten	30	1	.0418
	30	168	.0409
	70	1	.0407
	70	168	.0400

The results obtained with whole-wheat flour are similar to those reported for white flour (Shrader, Beshgetoor, and Stenger, 1941). Most of the absorbed methyl bromide escapes by volatilization and the remainder reacts to form inorganic bromide. The greater part of the bromide present half an hour after fumigation is already in the inorganic form.

Samples of wheat starch and gluten were simultaneously fumigated with 2.05 pounds of methyl bromide per 1,000 cubic feet for 12 hours in a drum at 88°F. The dosage of fumigant corresponded to 13.1 pounds per 1,000 pounds of product. The data are reported in Tables IV and V and Figures 2 and 3 and indicate that the gluten fraction of wheat retains more bromide than the starch. The opinion has occasionally been expressed that milled cereals retain appreciable quantities of bromide primarily because of their fine state of subdivision. The starch used in these experiments was ground as finely as the flour and much more finely than the gluten, yet it retained considerably less bromide. Evidently particle size is not the only determining factor in the absorption of bromide by cereals.

The effect of relative humidity on the absorption of methyl bromide during atmospheric fumigation has apparently not been studied previously. Unexplained differences in retentions of bromide obtained during summer and winter laboratory fumigations of flour led us to suspect that the humidity had some influence, so an experiment was performed to decide the question. Duplicate samples of each of several

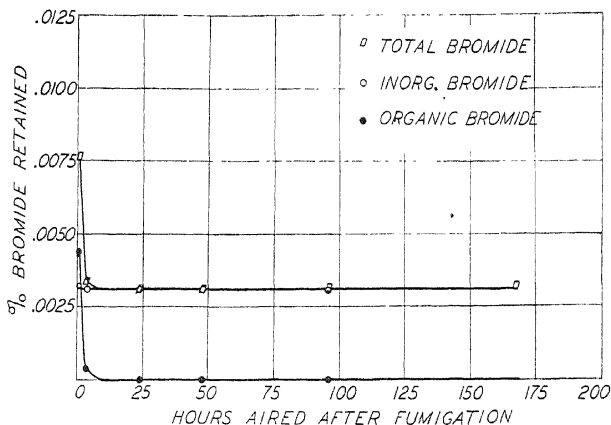


Fig. 2. Retention of bromide by wheat starch.

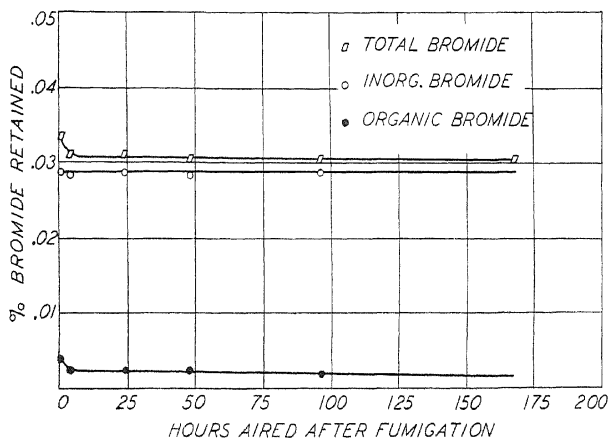


Fig. 3. Retention of bromide by wheat gluten.

wheat products were fumigated at two relative humidities in a 500-cubic-foot vault. The dosage in each case was two pounds of methyl bromide per 1,000 cubic feet, or 330 pounds per 1,000 pounds of product. Fumigation was continued for 24 hours at 80°F. The retentions, which naturally were higher than those obtained in commercial fumigations, are shown in Table IV.

Although the values appear rather confusing, they can be interpreted as being the result of two factors. First, water vapor is adsorbed preferentially to methyl bromide at the surface, so that with starch, for example, less methyl bromide is adsorbed at the higher

humidity. Second, adsorbed or absorbed methyl bromide hydrolyzes or reacts more rapidly in the presence of moisture. Thus the methyl bromide adsorbed on starch at 30% relative humidity escapes completely upon airing, whereas that taken up at 70% largely remains as inorganic bromide. With gluten, the bromide is taken up mainly by reaction and the surface displacement is almost wholly compensated by the increased reactivity under moist conditions. The effects noted with white and whole-wheat flour are intermediate between those observed with starch and gluten. It is of interest to realize that at low humidity more of the methyl bromide escapes by volatilization, while at high humidity more of it undergoes reaction.

Summary

It has been shown that the bromide retained by cereal products following fumigation with methyl bromide exists almost entirely in inorganic form after standing for a few days, and, furthermore, that the amounts retained are likely to be smaller in commercial fumigations than in laboratory experiments.

The retentions of bromide by different cereal products have been compared. Most commercial cereals retain only small amounts.

The influence of relative humidity on the retention of bromide by wheat products has been studied briefly. At higher humidities water displaces part of the methyl bromide from the surface, but the methyl bromide which is taken up reacts more rapidly to form inorganic bromide.

Literature Cited

- Cotton, R. T.
1941 Insect pests of stored grain and grain products. Burgess Publishing Co., Minneapolis, Minn., pp. 198, 206, 215.
- Dudley, H. C., Miller, J. W., Neal, P. A., and Sayers, R. R.
1940 Studies on foodstuffs fumigated with methyl bromide. Reprint 2215 from Public Health Reports 55: 2251-2282.
- Hawkins, L. A.
1938 Fumigation of dormant deciduous nursery stock with methyl bromide. Bureau of Entomology and Plant Quarantine, U. S. Dept. Agr., E-458.
1939 The use of methyl bromide for the treatment of quarantined plant products. Bureau of Entomology and Plant Quarantine, U. S. Dept. Agr., E-484.
- Laug, E. P.
1941 Bromide residues in foodstuffs. Ind. Eng. Chem. 33: 803-805.
- Mackie, D. B.
1938 Methyl bromide—its expectancy as a fumigant. J. Econ. Entomol. 31: 79-84.
- and Carter, W. B.
1937 Methyl bromide as a fumigant; preliminary report. Calif. Dept. Agr. Bull. 26: 153-162.
- Shrader, S. A., Beshgetoor, A. W., and Stenger, V. A.
1941 The determination of total and inorganic bromide in foods fumigated with methyl bromide. Ind. Eng. Chem. (Anal. Ed.), in press.
- Stenger, V. A., Shrader, S. A., and Beshgetoor, A. W.
1939 Analytical methods for methyl bromide. Ind. Eng. Chem. (Anal. Ed.) 11: 121-124.

EFFECT OF TEMPERATURE AND AGE ON MALT SYRUP

E. B. BROWN and B. H. NISSEN

Anheuser-Busch, Inc., Laboratories, St. Louis, Missouri

(Read at the Annual Meeting, May 1941)

In the commercial handling of malt syrup it sometimes happens that syrup while in the package appears to undergo a marked change resembling fermentation. The customer usually observes this condition several hours after having opened the package and accepted what appeared to be a perfectly smooth, dark-surfaced syrup, free of any indication of foam or fermentation whatever. Within a few hours after opening, however, copious foaming may appear and proceed even to the extent of foaming out of the container. The customer naturally assumes from both the appearance and the odor that this supply of malt syrup is fermenting and rapidly becoming unfit for use.

Like many others we have made frequent investigations of this particular phenomenon and its accompanying effect on the syrup, and it is of interest to present related information from the literature as well as a discussion of certain data and material gathered by ourselves. We observe that the darkening in color, and the formation of carbon dioxide accompanying this condition, is a reaction fairly common to many sugars. It has long been known that certain sugars in combination with some amino acids undergo a definite chemical reaction.

Neuberg and Kobel (1926) showed that fructose and *dl*-alanine react at room temperature under neutral conditions. Other sugars, glucose and maltose, were likewise tested not only with alanine but also with aspartic and gluconic acids and with arginine. Very striking changes in rotation were found with aspartic and gluconic acids, especially in the case of fructose.

Euler, Brunius, and Josephson (1926) found that a condensation occurs between glycine and glucose with equilibrium in 30 to 40 hours. The reaction is reversible, the compound gradually undergoing cleavage when the alkaline solution is acidified. Condensation between glucose and alanine occurs in the same manner. Preliminary treatment of the glucose with NaOH does not alter the reaction. At pH 9.5 the condensation amounts to as much as 53% in 48 hours. The system glucose-glycine-NaOH at pH 9.3 decolorizes methylene blue, whereas glucose-NaOH alone, at the same pH, has no effect.

Maillard (1912) demonstrated that when glycocoll is warmed with 4 parts of glucose in 3 to 4 parts of water the liquid slowly assumes first a yellow, then a dark brown color, followed by foaming (CO₂).

The oxygen of the CO_2 comes from the carboxyl (CO_2H) group of the glycoll. Assuming that this loss of CO_2 is accompanied by a union of the nitrogen with aldehyde carbon of the sugar, the glucose molecules (at least two in number) forming part of the new compounds must undergo dehydrations resulting in the appearance of double bonds or possibly rings. Various other amino acids react in the same way on glucose and similarly various sugars act in this way on glycoll: xylose and arabinose immediately, fructose, galactose, glucose and mannose quite rapidly, lactose and maltose slowly, and sucrose not at all for several hours and then only very slowly.

Ambler (1929) states that the course of the reaction between glucose and amino acids depends entirely on conditions of temperature, concentration, and time. At high temperatures as much as 10 mols of glucose per mol of amino acid may be destroyed but lowering of the temperature reduces the effect. In this reaction compounds of progressive complexity of the general class of melanoidins are formed by dehydroxylation. The melanoidins greatly increase the tendency to froth and also increase the color of the products.

In the manufacture of sweetened condensed milk it was early noticed that with the use of glucose instead of sucrose the product had a marked tendency to darken in storage. Thus Haradine (1933) noted that the development of the brown color of sweetened condensed milk was attributable to the formation of humin due to reactions between protein or amino acids and sugars. The preheating temperature used in the manufacture of sweetened condensed milk has little effect upon the brown coloration, while temperature of storage had marked effects on the rate of its development. High storage temperature (e.g. 100°F) promoted rapid development of the brown color, while at storage temperature below 68° it formed slowly. The use of invert sugar syrup as a substitute for cane sugar in condensed milk caused a brown coloration. It is suggested that the inversion of sucrose is responsible for the brown coloration, this not being evident to the eye until 25% of the sucrose has been inverted. The critical temperature at which the brown coloration develops is $80^\circ\text{--}90^\circ\text{F}$. The presence of iron salts was found to facilitate the development of color.

Our interest at first centered around the development of the foaming. In order to fully satisfy ourselves that, in the case of malt syrup, fermentation in no instance entered into the condition of such foaming syrup, innumerable samples were plated out at regular intervals. In no instance did we ever find that yeast had developed or that a usual yeast fermentation had taken place. Had yeast fermentation actually been the cause of the foam, the presence of

yeast cells in large numbers would certainly have been evident. Instead it will be shown that at temperatures well above pasteurization the formation of gas and foaming became progressively worse. In fact, we have often noticed that malt syrup either in cork-stoppered sample bottles or in other types of loosely stoppered containers never shows any foam nor any indication of sour odor or carbon dioxide whatever. In such containers fermentation could most easily take place were it possible. However, in malt syrup over 40° Bé it is doubtful that yeast growth could even exist.

In the experimental storage of samples of most malt syrups it was found that the syrup usually increased in color, especially if held at temperatures around 100°F. If the syrup was held in tightly sealed containers pressures of from a few ounces up to as high as 9 lbs were found to develop.

Since the literature refers to this relation between color and gas formation the following experiments were conducted. Various batches of different malt syrups were heated in different ways to study the effect of temperature on color, foam, protein and sugar.

Experiment No. 1: Three lots of 42° Bé syrup were heated in tubes at 165°F for 1 hour. A large amount of foam gathered on the surface of each sample.

Experiment No. 2: Malt syrup direct from process was heated in 600-ml beakers immersed in water baths in such a way that the syrup could be brought to the desired temperature in about 40 minutes, held at this temperature for 4-hour intervals, and then cooled and color determination made. It was then reheated for two more intervals of 4 hours each.

A great deal of foam formed on the surface of the 170°F beaker even to the extent of overflowing in 12 hours of heating. Foam formation was rather prominent in the 160°F beaker too, but only about a fifth as great as that of the 170°F sample. The 140°F and the 150°F beakers showed practically no foam in 12 hours' heating. The colors developed during this heating are given in the following table, the colors being on dilutions determined with the Lovibond Tintometer.

TABLE I
COLOR OF HEATED MALT SYRUP (42° Bé SYRUP)

	4 hours' heat	8 hours' heat	12 hours' heat
Control	8	8	8
140°F	9	10	11
150°F	10	12	13
160°F	11	14	19
170°F	14	21	31

Protein determinations were made on these various samples of malt syrup, but showed no change even for the 170°F-heated malt syrup.

The degree of fermentation was also noted on these variously heated malt syrups by making a dilution of the syrup to a definite degree Balling (11.0). The degree of fermentation appeared to be

TABLE II
PROTEIN IN HEATED MALT SYRUPS (42° BÉ SYRUP)

	Protein
	%
Control	6.38
140°F	6.30
150°F	6.30
160°F	6.30
170°F	6.38

inversely affected by the higher heating, decreasing as the temperature is increased. The results are shown in Table III.

TABLE III
DEGREE OF FERMENTATION—MALT SYRUP HEATED FOR 12 HOURS

	Degree
	%
Control	81
140°F	76
150°F	71
160°F	69
170°F	64

Figure 1 shows the amount of foam present in the various beakers after the 12 hours of heating.

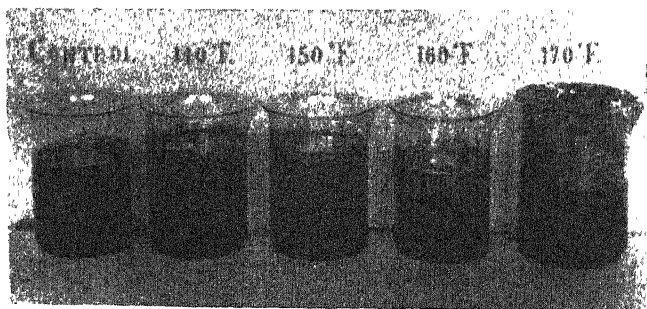


Fig. 1. Foam developed in 12 hours of heating.

Experiment No. 3: Light-density malt syrup from third effect vacuum pan (only 30° Bé) was heated in 600-ml beakers in water bath for 3 hours at the temperatures given below. Considerable

foam formed on the 190°F sample, less on the 180°F, and practically none on the others. Color, protein, and degree of fermentation are shown in Table IV.

TABLE IV
30° BÉ SYRUP FROM THIRD-EFFECT PAN HEATED THREE HOURS

	Color	Protein	Available degree of fermentation
		%	%
Control	5.5	5.4	90
150°F	5.5	—	—
165°F	7.0	—	—
180°F	11.5	—	—
190°F	16.5	5.6	76

In these tests it is quite evident that color and gas develop together and become more rapid and profuse as the temperature increases. Little change took place in the proteins but about 15% of the sugar value was lost at the higher heating. These experiments indicate, therefore, that temperature plays a definite role in the darkening of malt syrup and gas formation.

Tests were then made of ordinary room temperature storage samples held for various periods. Color, acid, and diastase were obtained when the malt syrup was first made and when later examined at the interval shown in Table V.

TABLE V
ANALYSIS OF 42° BÉ MALT SYRUP AFTER STORAGE AT ROOM TEMPERATURE
(Original color about 60, acidity 0.45% to 0.60% and diastase 40° to 45° Lintner)

Lot no	Time stored	Analysis after storage		
		Color	Acid	Diastase
	months		%	° L.
1	2	95	0.50	46
2	3	160	0.55	44
3	4	185	0.61	45
4	5	210	0.68	43
5	6	270	0.73	42
6	7	350	0.76	43
7	8½	430	0.75	47
8	9	485	0.83	44
9	10	545	0.86	45
10	11	560	1.05	48

The syrups showed a marked increase in color, some increase in acidity, and little if any change in diastase. Other storage tests were made also over longer periods of time, as shown in Table VI.

TABLE VI
ADDITIONAL TESTS ON 42° BÉ MALT SYRUP STORED AT ROOM TEMPERATURE

Age	Acidity as lactic	Color 1" cell
months	%	
3	0.88	148
6	0.99	198
9	1.41	363
12	1.68	836
15	1.84	1352
18	1.85	1453
21	1.90	1503
24	1.96	1653

Conclusions

The above tests indicate that the color of malt syrup is a function of temperature and time, and that foaming results if the samples are hermetically sealed during long storage at room temperature or more rapidly at high temperatures even if open to the air. Acidity increases slowly but definitely and diastase appears to have been little affected for the times studied.

It would appear from these tests, therefore, that malt syrup should always be kept rather cool and never placed near a hot bake oven. And that if it does foam the foaming is not due to fermentation but to a reaction which appears to be accelerated by warm-temperature storage and by failure to provide proper venting for the carbon dioxide which apparently results and which will dissolve in the syrup. When such a tightly sealed container is opened the dissolved carbon dioxide soon begins to escape, causing the malt syrup to foam over. Cool storage and pin hole venting should be the remedy for this occasional behavior of malt syrup.

Literature Cited

- Ambler, J. A.
1929 The reaction between amino acids and glucose. *Ind. Eng. Chem. (Anal. Ed.)* **21**: 47-50.
- Euler, H. v., Brunius, E., and Josephson, K.
1926. Über Reaktionen zwischen Zuckerarten und Aminen. II. Weitere Versuche mit Glucose und Aminosäure. *Z. physiol. Chem.* **155**: 259-269.
- Haradine, C. E.
1933 Inversion of sucrose in the manufacture of sweetened condensed milk. *Nat. Butter Cheese J.* **24**: No. 19, 7, 41; No. 20, 7, 16; No. 21, 16-20.
- Maillard, L. C.
1912 *Chimie organique. Action des acides amines sur les sucres; formation des melanoidines par voie methodique.* *Compt. rend.* **154**: 66-68.
- Neuberg, C., and Kobel, M.
1926 Weiters über die Reaktion einzelner Zuckerarten mit verschiedenen Aminosäuren nebst Bemerkungen über die Vergärung von Aminosäuren-Zucker-Gemischen. *Biochem. Z.* **174**: 464-479.

REPORT OF THE MALT ANALYSIS STANDARDIZATION COMMITTEE

ALLAN D. DICKSON, *Chairman*

U. S. Department of Agriculture and University of Wisconsin, Madison, Wisconsin

(Read at the Annual Meeting, May 1941)

Upon the recommendation of last year's committee (1940) the ferricyanide method for the determination of diastatic power was adopted as a tentative method. The committee also recommended that certain phases of the method be studied further and that existing methods for the determination of alpha-amylase be submitted to preliminary investigation. This latter study is in progress, but has not been completed.

In the interest of unifying the methods recommended by our organization and those of the American Society of Brewing Chemists, a joint collaborative study was made of the A.S.B.C. (1940) and ferricyanide methods for the determination of diastatic power in malt. The primary purpose was to verify the conversion factor for the ferricyanide method as given by Anderson and Sallans (1937) which would give Lintner values that agree with those obtained by the A.S.B.C. method.

Samples of malt flour and soluble starch were sent to nineteen collaborators. On the basis of the method used for routine work, ten laboratories were selected to report on the ferricyanide method and nine on the A.S.B.C. method. The malt flour was to be used at four concentrations (10, 15, 20 and 25 g per 500 ml) to give diastatic-power values of approximately 75° to 190° Lintner. Duplicate determinations were to be made at each concentration on two days.

Nine collaborators submitted results obtained with the ferricyanide method and seven with the A.S.B.C. method. Since the primary aim of this study was to determine a conversion factor for the ferricyanide method, the means of the A.S.B.C. values were used as bases and these divided by the mean net ferricyanide titrations to give conversion factor for the different concentrations. The average value was 22.8 with a variation from 22.6 to 22.9. This is in excellent agreement with the factor suggested by Anderson and Sallans, which is 22.5 where 2 ml of infusion is used with a final volume of 250 ml. For convenience, it is recommended that a factor of 23 be used in calculation when the above conditions of diastasis are used.

The means for from two to four determinations for each collaborator at each concentration and for the two methods are given in Table I. Where the factor of 23 is used the agreement between the two methods is excellent and the variations within the two methods are of approxi-

TABLE I

DIASTATIC POWER IN DEGREES LINTNER (DRY BASIS) OF FOUR CONCENTRATIONS OF MALT FLOUR BY THE A.S.B.C. AND FERRICYANIDE METHODS—MEANS OF 2-4 DETERMINATIONS

A.S.B.C. METHOD				
Collaborator	10 g	15 g	20 g	25 g
1	72	114	148	188
2	77	115	151	193
3	76	117	156	196
4	83 [*]	119	159	203
5	72	108	137 ¹	149 ¹
6	72	106	143	180
7	73	117	153	185
Average	74	114	152	191

FERRICYANIDE METHOD				
Collaborator	10 g	15 g	20 g	25 g
1	73	112	150	193
2	74	112	149	190
3	76	118	159	201
4	75	112	152	191
5	71	107	144	180
6	72	112	148	185
7	79	121	161	201
8	76	115	156	195
9	85 [*]	126	164	205
Average	75	115	154	192

* Starred values not included in the averages

mately the same magnitude. The average differences between duplicates and days, using the means for days and means for duplicates, were 1.1 for duplicates and 1.9 for days with the ferricyanide method. Similar values for the A.S.B.C. method were 1.3 and 1.8.

The committee recommends:

That a factor of 23 be used in calculating degrees Lintner from ferricyanide titration where the procedure of the A.S.B.C. method for diastasis is used.

That the results of the alpha-amylase study be published as soon as completed and that further study of the most promising methods be continued.

That close cooperation with the malt committee of the American Society of Brewing Chemists be continued on methods of mutual interest.

Acknowledgment

The chairman expresses his appreciation to the committee members and collaborators, whose willing cooperation made this report possible, and to R. I. Tenney and S. Jozsa of the American Society of Brewing Chemists for their cooperation and assistance in this study.

Literature Cited

- American Society of Brewing Chemists
 1940 Methods of analysis (3rd rev. ed.)
 Anderson, J. A., and Sallans, H. R.
 1937 Determination of the diastatic power of malt in degrees Lintner by means of a ferricyanide reagent. *Can. J. Research* C15 70-77.
 Dickson, A. D.
 1940 Collaborative study of the ferricyanide method for the determination of diastatic power of malt. *Cereal Chem.* 17: 645-649.

CHANGES IN THE BARLEY KERNEL DURING MALTING: CHEMICAL COMPARISONS OF GERM AND DISTAL PORTIONS ¹

ALLAN D. DICKSON and B. A. BURKHART

U. S. Department of Agriculture and University of Wisconsin, Madison, Wisconsin

(Read at the Annual Meeting, May 1941)

The chemical changes that occur during the malting of barley are of fundamental importance in the subsequent use of the malt. No attempt will be made here to review in detail the large number of studies which have been made of this subject. The changes in the activity of different enzymes during malting and kilning have been reviewed by Lüers (1936). In general, there is an activation or production of enzymes during the germination period, followed by a reduction during the kilning process. Ohlsson and Thörn (1938) followed the development of alpha-amylase during germination. This enzyme was practically absent until the third day of germination, when it increased rapidly, reaching its maximum in about five days.

These studies were made on the whole barley or malt kernels and gave an average picture of the changes within the whole kernel. Some early determinations were made by Brown and Morris, Windisch and Hasse, and Ling of the comparative amounts of diastase in the various sections of malt kernels. Windisch and Kolbach (1929) reviewed these and studied the total and cold-water-soluble nitrogen fractions in the germ and distal ends of barley and malt kernels after 15 days' germination. The total nitrogen was not greatly different in the two ends. In the barley there was somewhat more soluble nitrogen in the

¹ Cooperative investigations between the University of Wisconsin and the U. S. Department of Agriculture, Division of Cereal Crops and Diseases, Bureau of Plant Industry.

germ end than in the distal, and in the 15-day malt the soluble nitrogen in the germ end was from two to three times greater than in the distal end.

Histochemical or microchemical methods applied to microtome sections of material have been used to study barley and malt kernels by Linderstrom-Lang and others. Glick (1938) has applied this technique to studies on the ascorbic acid distribution in the developing embryo of the barley kernel, and gave his findings and a review of the findings of Linderstrom-Lang and Engel (1938) on the distribution of the amylase in the outer layers of the barley kernel. This technique provides valuable information but of necessity is applied to such small portions of the kernel that a complete picture of all the various changes taking place during germination requires many separate studies.

In the course of an investigation of malting methods there were available barleys and malts representing two to nine days of germination under various conditions. This material offered an excellent opportunity to obtain information on the chemical changes and enzyme distribution in the two halves of the kernels. A discussion of the changes in cellular structure with associated chemical changes during malting was given by Dickson (1940). The present paper represents a more detailed discussion of the chemical changes in the two portions of the kernels during germination.

Materials and Methods

Two varieties of barley, *Oderbrucker* (Wis. Ped. 5-1) and *Wisconsin Barbless* (Wis. Ped. 38) were malted at approximately 43% and 48% moisture at 16°C. *Oderbrucker* samples grown and malted in 1936 and 1937 and one sample of *Wisconsin Barbless* grown and malted in 1938 were used. Barley samples were steeped to the appropriate moisture contents and placed in the large malting unit at daily intervals for seven days. All were removed on the ninth day and given a uniform kilning treatment. This gave a series of malts grown from two to nine days under uniform conditions.

Samples of the barleys and the eight malts in each series were sectioned by hand into approximately equal halves. Samples of the sectioned portions and of the whole kernels were analyzed for moisture, total ash, total nitrogen, diastatic power, extract, and the various nitrogen fractions in the worts. Water extractions of the barleys and malts were made at 5°-7°C and total extract, total nitrogen, permanently soluble nitrogen, and formol nitrogen were determined on these extracts.

The methods of the American Society of Brewing Chemists (1937) were used for moisture and extract. Diastatic power was determined

by the ferricyanide method of Anderson and Sallans (1937). Total ash and nitrogen were determined by the methods of the Association of Official Agricultural Chemists (1935). Total wort nitrogen and cold-water-soluble nitrogen were determined on 25-ml aliquots. Permanently soluble nitrogen was determined on aliquots of the filtered sample after boiling for one hour under a reflux. Formol nitrogen was determined electrometrically with a glass electrode by adjusting a 25-ml aliquot to pH 9.0, adding formaldehyde, and titrating back to pH 9.0. Alpha-amylase determinations by the method of Blom and Bak (1938) were made on the Wisconsin Barbless series of 1938.

Presentation and Discussion of Data

Although the percentage of the germ and distal portions varied considerably from sample to sample as a result of variations in the cutting, the average values for quite a number of samples were 55% germ portion and 45% distal portion.

TABLE I

AVERAGE VALUES FOR TOTAL ASH AND TOTAL NITROGEN ON WHOLE KERNELS AND GERM AND DISTAL PORTIONS OF ODERBRUCKER AND WISCONSIN BARBLESS BARLEYS AND MALTS

	Whole-kernel barley	Distal end barley	Germ end barley	Mean of 2-9 day malts		
				Whole-kernel malt	Distal end malt	Germ end malt
	%	%	%	%	%	%
ODERBRUCKER						
Total ash	3.03	2.71	3.37	—	2.33	3.01
Total nitrogen	1.95	1.86	2.09	2.00	1.81	2.02
WISCONSIN BARBLESS						
Total ash	2.91	2.77	3.00	—	2.17	2.87
Total nitrogen	1.84	1.73	1.86	1.80	1.72	1.76

The large amount of data accumulated has been simplified in presentation by averaging values for the high and low moisture maltings of the 1936 and 1937 Oderbrucker samples. The data for Wisconsin Barbless are means of the high and low moisture malting series for one year, 1938. Where the effect of moisture is discussed, the values for each moisture are used. Most of the data are presented graphically to show the trends in the various constituents throughout the malting period.

Ash and Nitrogen

The percentages of total ash and nitrogen showed no consistent trend with malting time in either portion of the kernel so the data are presented in summary form in Table I, using the mean values for the eight malts. Ash was significantly higher in the germ ends of both barley and malt kernels than in the distal ends or in the whole kernels. Both ends of the barley kernels were higher in ash than the malt kernels, indicating an extraction of mineral materials during steeping and malting. The percentage loss from the distal portions was somewhat greater than from the germ portions.

The trends for nitrogen content in the two portions of the kernels were similar to those for ash, being slightly higher in the germ portion. This difference was less for Wisconsin Barbless than for Oderbrucker samples. The sectioned portions of the malt kernels were slightly lower in nitrogen than similar portions of the barley.

Diastatic Power

The data on diastatic power of the sectioned portions and the whole kernel of the two varieties are presented graphically in Figure 1. The characteristic difference between the two varieties in this factor is evident and accentuated in the germ portions of the kernels. The enzyme was activated more rapidly in Oderbrucker than in Wisconsin Barbless and a greater quantity was produced. The amylases

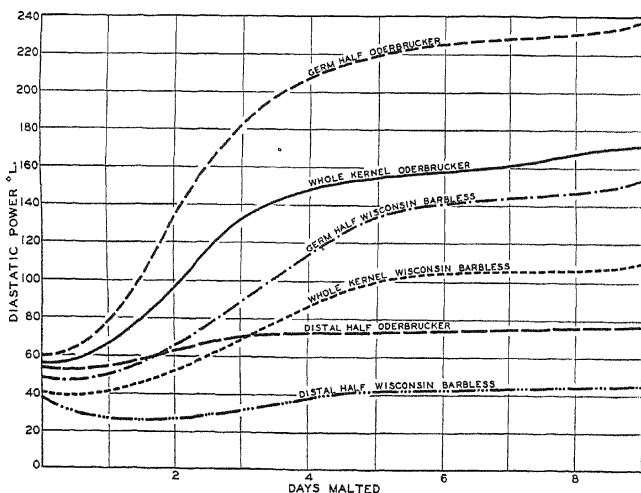


Fig. 1. The production of diastatic power in whole kernel and germ and distal portions of Oderbrucker and Wisconsin Barbless barley kernels during malting.

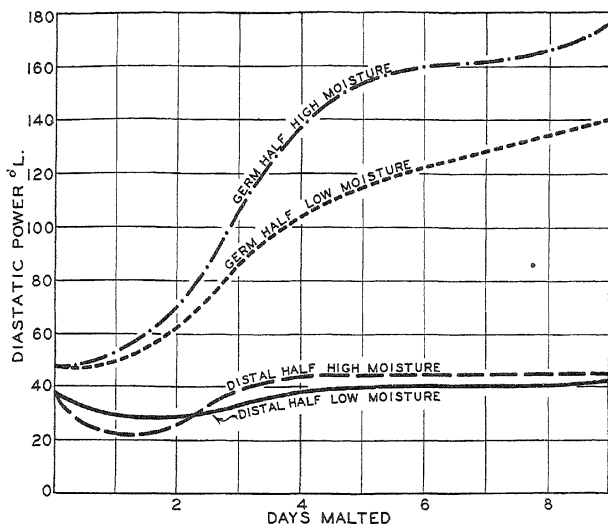


Fig. 2 The effect of moisture during malting on the diastatic power of germ and distal portions of malt kernels produced from Wisconsin Barbless barley

measured by the diastatic power method, considered to be primarily beta-amylase, were activated or produced and retained principally in the germ half of the kernel. In the distal portion of the Oderbrucker kernels, the increase in diastatic power from the barley to the 9-day malt was 43%. In the germ half the increase was 293%. For Wisconsin Barbless the corresponding values were 18% for distal and 230% for germ halves.

These data indicate that the amylases were not activated or produced as rapidly or to the same extent in the distal portion as in the germ half of the kernel. Furthermore, if a movement of amylase from the site of greatest concentration during malting may be considered likely, the transfer into the distal end was not great. In 9-day malt from both varieties approximately 80% of the diastatic power of the whole kernel was found in the germ end and 20% in the distal end. In this connection, Linderstrom-Lang and Engel (1938) state that the amount of beta-amylase concentrated just within the aleurone layer constitutes approximately 20% of the total amylase of the kernel.

Diastatic power increased very rapidly in the germ portion and whole kernel until the fifth day. The increase after this was slight until the eighth or ninth day when a further slight increase took place as shown in Figure 1.

The influence of malting moisture on the diastatic power of the sectioned kernels of Wisconsin Barbless is shown in Figure 2. Since

the majority of the enzyme was produced in the germ end, the effect of moisture was much greater in this portion of the kernel. This was indicated by a more rapid rate of activation and an appreciably higher final value in the higher-moisture malting. The curve for the low-moisture malting shows a gradual rise throughout the nine days with the rate of activation decreasing appreciably after the fifth day. In contrast to this the high moisture curve is very similar to those shown for the germ portions and whole kernels in Figure 1. At 43% moisture, the amount of water may have been a limiting factor and thereby may have retarded the normal diastatic activation during germination.

Alpha-Amylase

Alpha-amylase by Blom and Bak's viscometric method was determined only on the Wisconsin Barbless sectioned kernels. The data showing the effect of moisture on this factor are shown graphically in Figure 3. The shapes of these curves are similar to those presented in Figure 2 for diastatic power. Perhaps the production of alpha-amylase was less influenced by moisture than was diastatic power. Here again the high moisture produced the flattening of the curve for the germ ends at the 6-, 7-, and 8-day malting periods.

The method used indicated practically no alpha-amylase in the distal ends, but a small amount in the germ ends of the barley kernels.

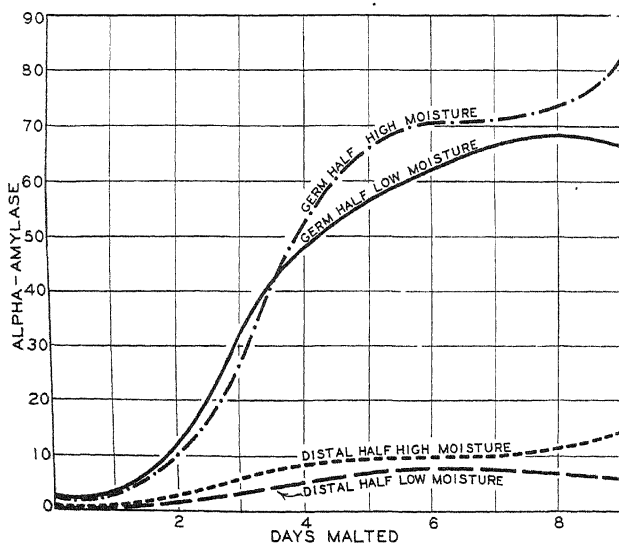


Fig. 3. The effect of moisture during malting on the alpha-amylase values for germ and distal portions of malt kernels produced from Wisconsin Barbless barley.

These values may have been caused in part by the presence of beta-amylase, since it has been shown to modify values obtained by this method. The small amounts of alpha-amylase in the barley were responsible for very large percentage increases from barley to 9-day malt. Using averages for the two moistures, the values for increase in alpha-amylase were 2,000% for the distal ends and 3,440% for the germ ends. This indicated that there was either a production of alpha-amylase in the distal end of the kernel or considerable diffusion from the germ end. However, at the end of 9 days of malting at high moisture approximately 85% of the alpha-amylase activity was in the germ half of the kernel. Therefore it appears that the distribution of alpha- and beta-amylase was approximately the same in the two halves of 9-day malt kernels. However, since there was practically no active alpha-amylase in the distal portion of the barley kernel, there was a much greater production of this component than of the beta component in the distal half during malting.

Soluble Nitrogen Fractions

The total cold-water-soluble nitrogen should be a measure of the amount of hydrolysis of proteins during malting. The extraction was made at a temperature of 5°-7°C in order to minimize the activity of the enzyme during extraction. On the other hand wort nitrogen is a measure of the amounts of hydrolysis occurring during malting and laboratory mashing. Wort nitrogen appears to be a fairly reliable indicator of proteolytic activity. In a series of malts representing different varieties furnished by this laboratory and reported upon by Koch, Nelson, and Ehrnst (1939), there was a very high correlation between the proteolytic power determined by their method and both the wort nitrogen and wort formol nitrogen. The data for total cold-water-soluble nitrogen and wort nitrogen, both calculated as percentage of barley or malt nitrogen, are presented graphically in Figure 4.

These curves show the trends in the nitrogen fractions in the two portions of the kernel and the whole kernels of the two varieties during malting. A marked similarity in the shapes of the curves for the two fractions is evident. As was true of the amylases, the activity of the proteolytic enzymes was consistently greater for Oderbrucker than for Wisconsin Barbliss. The difference between varieties was again greater in the germ than in the distal portions, although this was not so marked as with the amylases. In fact, when expressed as percentage of the amount of nitrogen present in the two portions, the difference between the varieties was about equal in the germ and distal halves.

It is apparent from the graphs that there was much greater enzymatic activity in the germ than in the distal portion. Considering the cold-water-soluble nitrogen, where it was possible to obtain this value on the barley sections, there was a 95% increase in the distal portion of the Oderbrucker from barley to 9-day malt. The corresponding increase for the germ portion was 176%. Similar values for Wisconsin Barbless were 114% for distal portions and 166% for germ

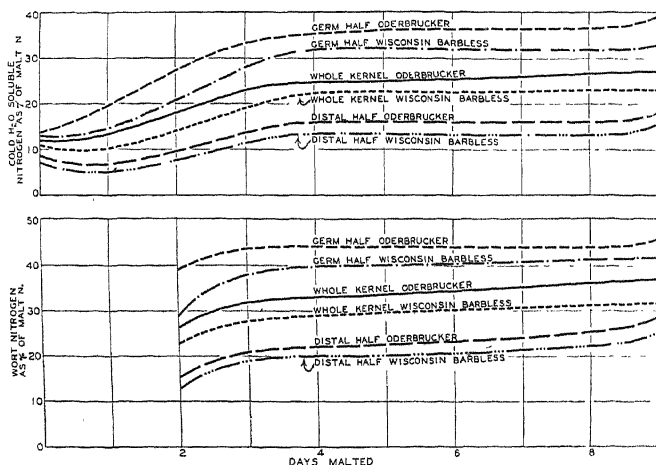


Fig. 4. The production of wort nitrogen and cold-water-soluble nitrogen in whole kernel and germ and distal portions of Oderbrucker and Wisconsin Barbless barley kernels during malting.

portions. The value for the germ half represented a doubling or less of the percentage increase in the distal half. In the case of the diastatic power, the percentage increase in the germ portion was 7 and 13 times that in the distal portion in Oderbrucker and Wisconsin Barbless, respectively. This indicates a much greater production or activation of the proteolytic enzymes in the distal ends of the kernels, or a fairly rapid transfer of these enzymes throughout the kernel during malting. Histological evidence, as presented by Dickson (1940), indicated that probably the latter was the case, since the increase in cold-water-soluble nitrogen was associated with the removal of the starch embedding matrix of the endosperm. The removal of the embedding materials started adjacent to the scutellum and progressed toward the distal end of the kernel as malting continued.

The data for cold-water-soluble nitrogen and wort nitrogen are presented in a different manner in Figure 5. Considering the cold-water-soluble-nitrogen as that solubilized during malting, the difference between the wort nitrogen and cold-water-soluble nitrogen represented

the additional amount of nitrogen made soluble during laboratory mashing. The cold-water-soluble nitrogen in the germ and distal portions of the barleys was subtracted from both values for the malts. The shaded portion of the diagram then represents the amount of nitrogen made soluble during malting, while the clear area under the upper curve represents the amount made soluble during laboratory mashing.

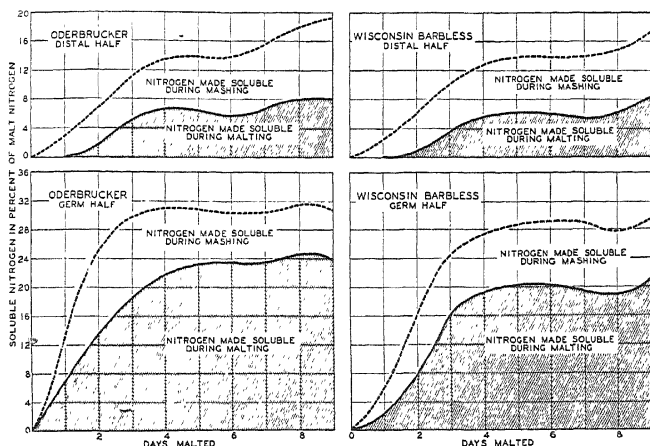


Fig. 5. Comparative amounts of total nitrogen made soluble during malting and mashing in germ and distal portions of Oderbrucker and Wisconsin Barbless malts.

In the distal portion of the Oderbrucker 9-day malt, 41% of the total amount of soluble nitrogen was made soluble during malting, leaving 59% made soluble during mashing. This ratio was practically the same for the 6-day malt. In the germ portion, even at 6 days, 80% of the nitrogen was made soluble in malting and only 20% was solubilized during mashing. This ratio was unchanged at 9 days. Wisconsin Barbless presented a somewhat different picture. In the germ end, the ratios were about the same in 6- and 9-day malts with 70% of the total soluble nitrogen made soluble during malting. In the distal ends at 6 days 42% of the nitrogen was solubilized during malting and at 9 days this value had increased to 47%. Wisconsin Barbless malts contained a smaller amount of soluble nitrogen than Oderbrucker and a smaller percentage of that nitrogen was made soluble during malting. Also in the distal end the nitrogen was made soluble during malting at a slower rate than in Oderbrucker, but eventually a greater percentage of the total was solubilized. This was further evidence of the greater enzymatic activity of Oderbrucker malt.

Permanently soluble and formol nitrogen were determined in the wort and in the cold-water extract. With possibly one exception, the correlation between these two factors and the total soluble nitrogen in the wort and in cold water extract was very high within any one kernel portion of a variety. Therefore, the data for these factors will not be presented graphically, as they should show the same relationships as wort nitrogen and cold-water-soluble nitrogen. Permanently soluble nitrogen indicated certain differences between the germ and distal portions of the malt kernels. In the laboratory worts, the permanently soluble nitrogen made up approximately 95% of the total wort nitrogen in both the germ and distal halves of the two varieties. This ratio remained fairly constant during the malting period. In the cold-water extract of the distal portions from the two varieties, the permanently soluble nitrogen constituted approximately 80% of the total cold-water-soluble nitrogen, and did not vary greatly from 2- to 9-day malts. In the germ portion, the permanently soluble cold-water nitrogen constituted a decreasingly smaller percentage of the total as malting progressed from 2 to 9 days. The average values for the 8 malts were 70% for Oderbrucker and 66% for Wisconsin Barbless. Considering the cold-water extract as an indication of the changes during malting, a smaller amount of the total nitrogen was permanently soluble in the germ end than in the distal portion, and this ratio became decreasingly less as malting progressed.

Laboratory and Cold-Water Extract

The data for laboratory extract on the germ and distal portions of the malt kernels and total cold-water extract on the barley and malt kernels are presented graphically in Figure 6. The presentation is similar to that used for the nitrogen fractions, the cold-water-extract values of the barley halves being subtracted from the malt values. The two fractions can then be looked upon as those made soluble in malting and mashing, respectively, but the values in the graph must be increased by approximately 8% to give the true extract values.

The distal halves of Oderbrucker kernels showed a higher laboratory extract value than those of Wisconsin Barbless, and the earlier activation of the amylases was indicated by the proportionately higher value for the 2-day malt. All of the values for the distal halves of 2- and 3-day malts are low because of insufficient enzyme to bring about conversion in one hour. The extract of the germ portions of Wisconsin Barbless malts was somewhat higher than for Oderbrucker, although the extract on the whole kernels was higher in the Oderbrucker.

In contrast to the soluble nitrogen fractions, the amount of extract made soluble in malting was a much smaller percentage of the total

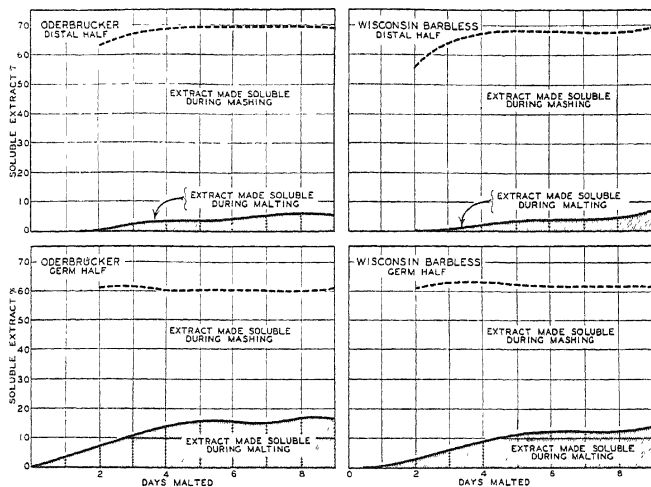


Fig 6. Comparative amounts of extract made soluble during malting and mashing in germ and distal portions of Oderbrucker and Wisconsin Barbless malts.

extract even in the germ portion. At the end of 9 days of malting 28% and 22% of the total extract were made soluble during malting in the germ portions of Oderbrucker and Wisconsin Barbless, respectively. Corresponding values for soluble nitrogen were 80% and 70%. The percentage of total extract made soluble in the distal portions of the two varieties did not differ greatly after 6 days. Approximately 6% was soluble at 6 days and this value increased to 9% at 9 days of malting. This appears to be further evidence that the proteolytic enzymes are distributed throughout the kernel much more completely than the amylases. In the case of the latter most of the hydrolysis of the starch takes place during mashing, after the material has been ground and the enzyme made available. Microscopical evidence presented by Dickson (1940) indicated very little decomposition of the starch grains even in 9-day malt.

Summary

The kernels of barleys and malts representing 2 to 9 days' germination of these barleys were sectioned into approximately equal halves. These were submitted to analysis with particular emphasis on the enzymatic changes taking place during germination. The data are presented graphically showing trends for the various factors during malting.

Total ash and nitrogen were reduced by steeping but did not vary appreciably during malting. Both were higher in the germ portion,

the difference being greater for ash than for nitrogen. Diastatic-power determinations indicated that the majority of the beta-amylase production or activation was in the germ half of the kernel and remains there. Alpha-amylase showed somewhat wider distribution throughout the kernel than the beta-amylase.

The proteolytic enzymes, measured by cold water and wort nitrogen, appeared to be distributed much more widely throughout the kernel than either of the amylases. From 70% to 80% of the total protein hydrolysis took place during malting in the germ half and only 20% to 30% during mashing. Carbohydrate hydrolysis during malting was comparatively low, in the germ end, attaining 20% to 25% of the total. This is interpreted as indicating contrasting activation and distribution of the two groups of enzymes during malting.

Acknowledgments

Acknowledgment is given to J. G. Dickson and H. L. Shands for production of the materials used in this investigation and for advice and assistance in the preparation of the manuscript. Eugene Herrling is given credit for the preparation of illustrative material. Assistance in the preparation of these materials was furnished by the personnel of the Works Projects Administration Project No. 65-1-53-2349.

Literature Cited

- American Society of Brewing Chemists
1937 Methods of analysis (2nd rev. ed.).
- Anderson, J. A., and Sallans, H. R.
1937 Determination of the diastatic power of malt in degrees Lintner by means of a ferricyanide reagent. *Can. J. Research* C15: 70-77.
- Association of Official Agricultural Chemists
1935 Methods of analysis (4th ed.).
- Blom, J., and Bak, A.
1938 Über die Bestimmung von Amylasen durch Verflüssigung von Starkekleister. *Z. physiol. Chem.* 256: 197-207.
- Dickson, J. G.
1940 Cellular modification and other changes in the barley kernel during malting. *Proc. 37th Ann. Convention Master Brewers Association of America*, pp. 29-40.
- Glick, D.
1938 Histochemical studies of the constitution of barley and malt. *Communications Wallerstein Lab.* 3: 7-14.
- Koch, J. R., Nelson, O., and Ehrnst, L.
1939 Viscometer for routine determination of proteolytic activity of malts. *Ind. Eng. Chem. (Anal. Ed.)* 11: 35-41.
- Linderstrom-Lang, K., and Engel, C.
1938 Über die Verteilung der Amylase in der äusseren Schichten des Gerstenkornes. *Compt. rend. trav. lab. Carlsberg, Ser. chim.* 21: 243-258.
- Lüers, H.
1936 Changes in enzymes during malting. *Cereal Chem.* 13: 153-171.
- Ohlsson, E., and Thörn, N.
1938 Amylases in resting and germinating seeds. V. The development of alpha-amylase in germinating barley. *Compt. rend. trav. lab. Carlsberg, Ser. chim.* 22: 398-404.
- Windisch, W., and Kolbach, P.
1929 Über die Verteilung des Gesamtpstickstoffs, des löslichen Stickstoffs und des koagulierbaren Stickstoffs auf das Spitzen- und des Keimende des Gersten- und Malzkorns. *Woch. Brau.* 46: 459-463.

COLLABORATIVE STUDY OF THE AYRE AND ANDERSON METHOD FOR THE DETERMINATION OF PROTEOLYTIC ACTIVITY¹

F. C. HILDEBRAND

General Mills, Inc., Research Laboratories, Minneapolis, Minnesota

(Read at the Annual Meeting, May 1941)

A previous study by the author (1940) compared the Landis and Frey rate of gelation and the trichloroacetic acid methods for the determination of proteolytic activity. While this study was based entirely on malted wheat flours as the experimental material, it was believed that results obtained could be applied equally well to other cereal products. In the study cited above, it was found that both methods give essentially similar results and that they have almost exactly the same experimental error and ability to differentiate between samples. The trichloroacetic acid technique, as given by Ayre and Anderson (1939), was preferred because of its greater simplicity and convenience. In this procedure, duplicate samples of the material to be tested are autolytically digested at 45°C for one- and three-hour periods. Following digestion, trichloroacetic acid is added to precipitate residual protein, and after filtration, determinations of soluble, *i.e.* nonprotein, nitrogen are made on aliquots of the respective filtrates. Proteolytic activity is calculated from the increase in nonprotein nitrogen from one to three hours' digestion.

In order to test the applicability of this method to materials other than malted wheat flour and to obtain an estimate of its reliability when applied in different laboratories, a collaborative study of the utility of the method was organized.

Experimental

Ten collaborators² tested a total of five samples. Included in the samples were a patent and a clear flour milled from a southwestern winter wheat; two samples of barley malt, one of high and one of low diastatic activity; and a sample of commercially produced malted wheat flour. The samples of barley malt were ground to pass a 100-mesh screen, and all samples were blended thoroughly by mechanical means. Subsamples were taken and immediately sealed and shipped to the several collaborators. The collaborators analyzed in duplicate the five

¹ Paper No. 28, Journal Series, General Mills, Inc., Research Laboratories Subcommittee report, 1940-41 Committee on Methods of Analysis.

² The author is indebted to W. G. Artis, G. S. Bratton, George Burkert, B. A. Burkhart, W. F. Geddes, R. H. Harris, Quick Landis, Stephen Laufer, L. E. Leatherock, and W. O. S. Meredith for their generous collaboration in this project

samples submitted with the results shown in Table I. In order to facilitate comparisons, an analysis of the data has been made, as shown in Tables II, III, and IV.

TABLE I
COLLABORATIVE DATA ON MEASUREMENT OF PROTEOLYTIC ACTIVITY
BY THE AYRE AND ANDERSON METHOD

Sam- ple	Collaborator										
	A	B	C	D	E	F	G	H	J	K	Av
1	82 84	77 69	137 153	96 92	109 119	115 94	103	92	71 94	114 105	100.0
2	221 231	197 160	127 122	257 255	285 234	247 244	310	249	242 271	198 198	230.4
3	254 264	221 360	165 159	294 300	299 321	297 300	310	292	270 276	225 229	271.9
4	65 55	87 33	67 72	63 66	49 35	39 39	140	0	35 32	53 51	49.0
5	264 274	180 164	162 172	261 263	253 279	263 274	211	225	262 242	242 246	233.6
Av. all samples	179.4	154.8	133.6	194.7	198.3	191.2	214.8	171.6	179.5	166.1	177.0

Figures represent mg nonprotein N per 100-g sample.

TABLE II
SUMMARY OF COLLABORATIVE DATA—EIGHT COLLABORATORS

VARIATION BETWEEN SAMPLES								
Sample No.	1	2	3	4	5			
Mean activity, as mg NPN/100 g	107.4	232.6	282.3	56.0	253.4			
	$F^1 = 56.9^\dagger$	1% pt = 4.57						
VARIATION BETWEEN COLLABORATORS								
Collaborator	A	B	C	D	E	F	J	K
Mean activity, as mg NPN/100 g	179.4	154.8	133.6	194.7	198.3	191.2	179.5	166.1
	$F^2 = 12.5^\dagger$			1% pt = 3.12				
Interaction $S \times C$, $F^2 = 6.30^\dagger$ 1% pt = 2.24								
Standard error of single determination = 19.8 mg.								

F^1 = interaction variance taken as error.

F^2 = duplicate variance taken as error

† Highly significant.

TABLE III
SUMMARY OF COLLABORATIVE DATA—FIVE COLLABORATORS

VARIATION BETWEEN SAMPLES					
Sample No.	1	2	3	4	5
Mean activity, as mg NPN/100 g	95.6	248.1	287.5	47.8	263.5
	$F^1 = 357.2\dagger$	1% pt = 4.77			
VARIATION BETWEEN COLLABORATORS					
Collaborator	A	D	E	F	J
Mean activity, as mg NPN/100 g	179.4	194.7	198.3	191.2	179.5
	$F^2 = 5.51\dagger$	1% pt = 4.18			
Interaction $S \times C$, $F^2 = 2.40^*$ 5% pt = 2.06 1% pt = 2.81					
Standard error of single determination = 11.8 mg.					

F^1 = interaction variance taken as error.

F^2 = duplicate variance taken as error

* Significant.

† Highly significant.

TABLE IV
COMPARISON OF STATISTICAL CONSTANTS—EIGHT AND FIVE COLLABORATORS

Variation due to	Eight collaborators	Five collaborators
	F	F
Between samples	56.9†	357.2†
Between collaborators	12.5†	5.51†
Interaction $S \times C$	6.30†	2.40*
Standard error of single determination	19.8 mg	11.8 mg

* Significant.

† Highly significant.

Discussion

In analyzing the data, the values submitted by collaborators G and H have been omitted since these individuals did not submit duplicate results. It will be seen that the method is adequate to distinguish between materials with as wide a range of proteolytic activity as was exhibited by the samples submitted. In addition, while the precision of the method is not as high as that of well standardized and common cereal chemical techniques, the results obtained by all collaborators compare favorably with data previously given by Ayre and Anderson. These authors report obtaining a precision of 18.6 units (standard error of a single determina-

tion) compared with the value of 19.8 obtained in the present study. It is indeed surprising that as high a level of precision was obtained in view of the fact that approximately half of the collaborators had not had previous experience with this technique.

Despite this relatively high precision, there are significant differences between laboratories and a significant tendency for the various collaborators to obtain relatively different results with the several samples. In general, however, the data indicate that this technique can be used by different laboratories with the expectation of obtaining fairly concordant results. If the method is to be applied extensively, it might be desirable to expend further effort to determine the cause or causes of the observed inter-laboratory variability. It has been suggested that one possible source of difficulty may lie in the use of containers of different wall thickness, which would cause different rates of heat transmission to the digestion mixtures.

As a matter of interest, the data shown in Table I were re-analyzed, omitting the data of the three collaborators B, C, and K. The results of this second analysis are shown in Table III, and the two are compared in Table IV. It will be seen that the inclusion of data from only five collaborators materially improves the differentiation between samples and the apparent precision of the method, while at the same time reducing the variability between collaborators and also reducing the magnitude of the interaction variation. These comparisons indicate that there is a distinct possibility of obtaining satisfactory results by the use of the Ayre and Anderson technique. It is believed that the improvement in results shown in the second analysis is due to the greater experience of the five collaborators with this method although it is possible that the difference is purely fortuitous.

Summary

The Ayre and Anderson trichloroacetic acid precipitation method for the determination of proteolytic activity has been studied collaboratively. Ten collaborators have applied the method to samples of a patent and a second clear flour, two of barley malt, and a malted wheat flour.

The level of precision attained appears to be satisfactory. The standard error of a single determination was found to be 19.8 mg nitrogen per 100 g, as against a comparable value of 18.6 mg given by Ayre and Anderson (1939). Significant tendencies were found, however, for different laboratories to obtain different results and for the several laboratories to obtain results varying with the different samples.

Literature Cited

- Ayre, C. A., and Anderson, J. A.
1939 Varietal differences in barleys and malts. VI Autolytic proteolytic activity of malt and its correlations with wort nitrogen and barley nitrogen fractions. *Can. J. Research* 17C: 239-246.
- Hildebrand, F. C.
1940 Comparison of methods for the determination of proteolytic activity. II Studies on malted wheat flours. *Cereal Chem.* 17: 635-639.

SOME PRELIMINARY STUDIES PERTAINING TO THE OPERATION AND USE OF THE BUHLER MILL

H. W. BAKER and N. L. GREGORY

Maple Leaf Milling Co., Ltd., Port Colborne, Ontario, Canada

(Read at the Annual Meeting, May 1941)

We have assumed that the primary purpose of the Buhler mill is to produce a flour from any particular sample of wheat, so that by means of interpretation and correlation the milling, blending, and baking values of the wheat may be determined prior to its being milled on the commercial unit. An exact duplication of the commercial flour with respect to flour yield, color, granulation, ash content, baking quality, etc. did not appear to be possible although it did seem probable that sufficient characteristics could be correlated to give the essential information. Since our main interest was to secure a knowledge of flour quality, we attempted to match the straight-grade experimental flour with the commercial flour of a similar extraction. At the same time, correlations with respect to milling characteristics were also desirable.

It must be pointed out that the scope of our experiments has been limited to our local conditions and to the use of Canadian spring wheats. These wheats were drawn from unload shipments at our own elevator, none of which therefore was of "pure variety" but consisted of the statutory grades from No. 1 Manitoba Northern to No. 4 Manitoba Northern, special grades (rusted or otherwise shrunken), and Garnet (a very vitreous variety). The data relative to the correlations between the Buhler and a commercial unit were obtained by using a 1500-barrel mill.

Buhler Mill Flow

The flow of our Buhler mill is similar to that outlined by J. E. Anderson¹ with the exception that a 10XX flouring silk is used on the first break section instead of 9XX and a 46 GG scalp replaced the 40 GG on the first reduction section.

¹ J. E. Anderson: Comparison of experimental and commercial milling results, *The Northwestern Miller* (Production No.), August 10, 1938, pp. 48-53.

Mechanical features of the mill: There are three break sections and three reduction sections. The three breaks are built on one pair of rolls, the first break having 16 corrugations per inch, the second 21 corrugations, and the third 26 corrugations.

The three reductions are likewise built on one pair of rolls which are smooth surfaced. The speed of the fast roll is 540 rpm and the differential is 2 : 1.

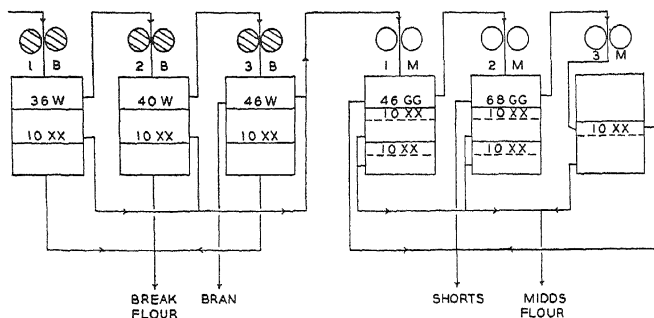


Fig. 1. Buhler mill-flow.

Wheat Conditioning or Temper

In order that flour yields from wheats of varying moisture content may be compared, a correction to a 13.5% moisture basis is made on the original sample of wheat when weighed into the mill.

The amount of moisture contained by the wheat and the distribution of this moisture at the time of grinding appear to have a definite effect on the milling conditions. The following method of tempering was adopted with good results. The wheat is scoured "as received" and then tempered to 16.0% moisture and allowed to stand for 24 hours. The added water may be applied with an atomizer or pipette and the sample thoroughly shaken up in a tin container. The sample is then scoured a second time before grinding with no further temper. To simplify the procedure, 2,100 g of wheat is weighed out on the basis of 16% moisture. An original sample of 2,100 g is used to allow for the loss in scouring, and for a moisture sample to be taken.

Example:

Moisture content of wheat "as received" = 10%. Weight of wheat at 10% moisture on basis of 2,100 g at 16% = $2,100 \times \frac{100 - 16}{100 - 10}$ = 1,960 g. Water (ml) required to raise 1,960 g wheat to 16% moisture = $2,100 - 1,960 = 140$.

After the second scour 2,060 g of wheat is weighed out $\left(2,000 \times \frac{100 - 13.5}{100 - 16} \right)$ and the yield is computed on 2,000 g at 13.5% moisture basis. It will be noted that no second temper immediately prior to milling is employed. This is explained by the fact that the clean-up of the bran is visibly affected. It was found that a second temper immediately prior to milling, sufficient to raise the moisture content of the wheat by 1%, increased the yield of bran by as much as $1\frac{1}{2}\%$ and decreased the yield of break flour by a like amount, without significantly affecting the ash content of the flour.

In milling the more vitreous wheats, a temper of 17% to 17.5% moisture is used. An "invisible" loss to the extent of 0.5% moisture usually occurs during the 24-hour temper. Milling tests of a commercially tempered wheat and of the same wheat experimentally tempered failed to show any significant difference in results.

It is our conclusion that for successful milling on the Buhler, the wheat must be thoroughly mellowed by adding sufficient moisture in the first temper. A second temper immediately prior to milling appears to cause a stickiness in the bran and prevents a satisfactory clean-up.

Grinding

Under the commercial system, wheats of varying weight per bushel or size of kernels would require a variance of roll settings on the first break. With the Buhler, however, the first-break roll is set closely enough to thoroughly break up the wheat kernel. Very little adjustment of roll setting, therefore, is necessary when one is grinding either a 60-lb or a 56-lb wheat. In order to obtain a knowledge of the operation of the Buhler, the character of the stocks and the type of flours that can be obtained, numerous samples of wheat of varying condition and weight per bushel were milled under a series of conditions. These included variations in temper, roll settings, and rate of feed. Hand sifting and chemical analysis of all the mill stocks gave an approximate idea of the percentage separations. These results are shown in Table I. As a rough check on the accuracy of Table I, Table II shows the comparative percentage separations by the milling test and by the hand-sifting method.

Since there is no classification of middlings, and because of the short reduction system, a partial reduction of the middlings must be accomplished on the break rolls. Approximately 70% of the first-break stock, a considerable amount of which is coarse middlings, is scalped over to the second break. Some of these middlings are reduced and 55% of the total break flour is obtained from the second

TABLE I
BUHLER MILLING DATA—HAND-SIFTED STOCKS

Flour	Roll	First break	Separation			Moisture	Ash	Protein
	%	%		%	%	%	%	%
1st break	2000	100	Over 36W	1400	70	14.8	1.86	15.0
			Over 10XX	480	24	14.2	0.56	12.4
			Thru 10XX	120	6	14.1	0.47	14.9
2nd break	1400	70	Over 40W	490	35	13.9	3.50	16.8
			Over 10XX	700	50	14.4	0.54	12.7
			Thru 10XX	210	15	14.7	0.45	16.3
3rd break	490	24.5	Over 46W	343	70	13.7	4.80	17.1
			Over 10XX	98	20	13.5	1.70	15.4
			Thru 10XX	49	10	13.6	0.76	19.2
1st midds	1278	63.9	Over 46GG	102	8	14.0	1.86	13.6
			Over 10XX	396	31	12.9	1.09	13.3
			Thru 10XX	780	61	13.9	0.40	12.8
2nd midds	396	19.8	Over 68GG	59	15	12.6	3.33	16.0
			Over 10XX	111	28	12.5	1.78	14.7
			Thru 10XX	226	57	12.6	0.52	12.5
3rd midds	111	5.5	Over 10XX	48	43	12.5	2.16	14.8
			Thru 10XX	63	57	11.4	0.82	13.5
Break	—	—	—	379	18.95	13.9	0.52	16.6
Midds	—	—	—	1069	53.45	13.0	0.465	12.7
Straight grade	—	—	—	1448	72.40	13.4	0.48	13.6
Shorts	—	—	—	209	10.45	11.9	2.60	15.0
Bran	—	—	—	343	17.15	13.7	4.80	17.1
Yield	—	—	—	—	72.40 = 4 bu, 30 lbs, 11 oz per barrel (Imperial measure)			

TABLE II

COMPARATIVE PERCENTAGE SEPARATIONS BY THE MILLING TEST AND BY THE HAND-SIFTING METHOD

Roll settings:	1st break	10.0	1st reduction	6.6
	3rd break	3.8	3rd reduction	3.2
Grinding time:	20 minutes			
Feed gate:	5 (scale reading)			
Size of sample:	2,000 grams			
	Milling test		Hand-sifted stocks	
	%		%	
Break flour	17.15		18.95	
Midds flour	54.75		53.45	
Total flour	71.90		72.40	
Shorts	7.00		8.45	
Bran	20.75		19.15	
Total feed	27.75		27.60	
Total products	99.65		100.00	
Loss	0.35		0.00	
Yield	4.31.12		4.30.11	

break. The third-break stock is mainly bran (3.5% ash) and 70% of this is tailed over to bran. Although the ash content of the third-break flour is rather high, it is not thought necessary to exclude it from the total flour.

The first-middlings stock, being made up of the second separation of all three breaks, contains middlings of varying size, the largest of which are the throughs of 36W and the smallest the tailovers of 10XX silk. This stock is made up approximately as in Table III.

TABLE III
COMPOSITION OF STOCK TO FIRST MIDDLINGS ROLL

Stock	Percent of mids stock	Ash	Protein
	%	%	%
1st mids	100	0.65	13.0
1st break over 10XX	37½	0.56	12.4
2nd break over 10XX	55	0.54	12.7
3rd break over 10XX	7½	1.70	15.4

Most of the middlings flour must be made on the first reduction section so that the tail of the mill is not overloaded. This requires very close grinding and results in some of the larger-sized middlings being flaked and scalped off to shorts by the 46 GG. However, there appears to be no alternative, for if the 46 GG is removed so that the flaked middlings may be reground on the second middlings roll, some of the bran particles which are present in the same stock are almost sure to appear in the second middlings flour. On the other hand, a finer scalp on first break tends to upset the balance of the mill. The

TABLE IV
ANALYSIS OF BREAK FLOUR

Flour	Percent of total flour	Percent of break flour	Ash	Protein
	%	%	%	%
Break	26	100	0.52	16.6
1st break	8.3	32	0.47	14.9
2nd break	14.5	55	0.45	16.3
3rd break	3.3	13	0.76	19.2

ANALYSIS OF MIDDLINGS FLOUR

Flour	Percent of total flour	Percent of mids flour	Ash	Protein
	%	%	%	%
Mids	74	100	0.46	16.6
1st mids	53.8	73	0.40	12.8
2nd mids	15.6	21	0.52	12.5
3rd mids	4.5	6	0.82	13.5

question has arisen as to whether a finely "scratched" or "figured" roll on first reduction would help offset the flaking of the larger middlings. The roll settings as shown in Table II were found to be most suitable for the reduction of the various stocks. Little adjustment is considered necessary for grinding wheats of different weight per bushel, apart from reducing the rate of feed for lighter-weight wheats and *vice versa*. The percentage of separation of the flours is shown in Table IV.

TABLE V
REPLICATE MILLINGS

2° WHEAT												
Wheat protein	Flour protein	Ash	GP ¹	Break flour	Midds flour	Total flour ²	Shorts	Bran	Total feed	Total production	Wt/bu ³	Yield
%	%	%		%	%	%	%	%	%	%	lbs	bu/bbl
14.2	13.5	0.45	420	17.75	53.40	72.20	6.65	20.90	27.55	98.70	63½	4.31.8
	13.5	0.46	404	17.95	53.75	72.55	7.00	20.10	27.10	98.80	—	4.30.0
	13.7	0.46	428	17.90	52.90	71.80	6.90	20.90	27.80	98.60	—	4.33.0
	13.5	0.45	420	17.70	52.90	71.50	7.30	20.80	28.10	98.70	—	4.34.0
	13.6	0.46	440	17.45	53.55	71.55	7.70	20.00	29.20	99.20	—	4.33.12
Av.	13.55	0.456	—	17.75	53.50	71.92	7.11	20.54	27.95	98.80	—	4.32.8
Standard deviation				0.20	0.39	0.45	0.40	0.45	0.78	0.23	—	—

3° WHEAT												
Wheat protein	Flour protein	Ash	GP ¹	Break flour	Midds flour	Total flour ²	Shorts	Bran	Total feed	Total production	Wt/bu ³	Yield
%	%	%		%	%	%	%	%	%	%	lbs	bu/bbl
12.6	11.8	0.45	580	18.15	50.25	69.30	8.50	21.75	30.25	98.65	63	4.43.0
	11.9	0.45	596	18.00	50.70	68.50	9.65	22.10	31.75	100.45	—	4.46.0
	11.9	0.45	576	17.80	49.40	69.60	7.40	22.00	29.40	96.60	—	4.42.0
	11.7	0.45	576	17.85	50.30	68.90	9.00	21.90	30.90	99.05	—	4.44.8
	11.8	0.44	600	17.90	51.85	69.75	9.15	21.15	30.30	100.05	—	4.41.0
Av.	11.8	0.448	—	17.94	50.50	69.21	8.74	21.78	30.52	98.96	—	4.43.0
Standard deviation				0.16	0.91	0.51	0.85	0.38	0.87	1.50	—	—

4° WHEAT												
Wheat protein	Flour protein	Ash	GP ¹	Break flour	Midds flour	Total flour ²	Shorts	Bran	Total feed	Total production	Wt/bu ³	Yield
%	%	%		%	%	%	%	%	%	%	lbs	bu/bbl
13.6	12.8	0.49	540	16.65	50.65	67.60	10.60	21.65	32.25	99.55	61	4.50.0
	13.0	0.49	552	16.40	50.15	67.10	10.90	21.90	32.80	99.35	—	4.52.0
	12.8	0.48	528	16.55	50.20	67.30	10.10	22.25	32.35	99.10	—	4.51.0
	12.7	0.48	526	16.35	51.65	68.90	9.65	21.15	30.80	98.80	—	4.44.8
	12.8	0.48	540	16.30	51.80	68.40	10.00	21.50	31.50	99.60	—	4.46.8
Av.	12.8	0.484	—	16.45	50.89	67.86	10.25	21.69	31.94	99.18	—	4.48.8
Standard deviation				0.15	0.79	0.76	0.42	0.41	0.79	0.33	—	—

ANALYSIS OF VARIANCE

	Sum of squares	Degrees of freedom	Variance	F
Between grades	42.75	2	21.374	61.2
Error	4.187	12	.349	—
Total	46.937	14	—	—

¹ GP = gassing power (Sandstedt and Blish, Cereal Chem. 11: 381-2, 6 hours, 30°C).

² Total flour—computed on basis of total products.

³ Wt/bu = weight per bushel—Imperial measure.

Duplication of Results

The factors which might cause variability probably exist within the mill itself and/or are a result of the technique of the operator. To determine the possibility of maintaining uniform milling conditions

TABLE VI
RANDOM MILLING RESULTS SHOWING EFFECT OF PHYSICAL CONDITION OF
WHEATS WITHIN GRADES

2° WHEATS							
Wt/bu ¹	Wheat protein	Flour protein	Ash	GP ²	Total ³ flour	Total feed	Yield
<i>lbs</i>	%	%	%		%	%	<i>bu/bbl</i>
62½	14.2	13.7	0.48	438	72.20	27.80	4.31.8
63	14.3	13.4	0.45	446	72.50	27.75	4.30.8
63	14.3	13.5	0.46	450	72.70	27.55	4.29.8
63	14.3	13.7	0.45	380	72.80	28.70	4.29.0
62½	14.2	13.6	0.47	380	72.85	27.25	4.29.0
62½	14.1	13.3	0.46	436	72.15	27.90	4.31.8
62	13.9	13.2	0.46	342	72.00	28.38	4.32.0
63	13.7	13.2	0.46	354	71.75	28.15	4.33.0
62	14.2	13.7	0.47	444	73.10	26.60	4.28.0
62½	14.2	13.4	0.47	400	72.30	27.50	4.31.0
Av.	14.1	13.5	0.463	—	72.43	27.76	—
Standard deviation	—	—	—	—	0.41	0.59	—

3° WHEATS							
Wt/bu ¹	Wheat protein	Flour protein	Ash	GP ²	Total ³ flour	Total feed	Yield
62	13.9	13.4	0.47	360	70.60	29.35	4.37.8
61	14.1	13.7	0.47	438	69.80	30.00	4.41.0
62	13.8	13.3	0.45	424	71.60	28.40	4.33.8
62	13.1	12.5	0.45	508	71.00	29.40	4.36.0
62	12.8	12.0	0.46	530	69.70	30.10	4.41.0
61	14.1	13.3	0.45	390	70.10	29.80	4.39.8
61	13.2	12.5	0.46	364	70.40	29.50	4.38.0
61	13.1	12.2	0.47	520	70.25	29.75	4.39.0
61	15.7	14.9	0.46	500	72.10	28.00	4.32.0
62	14.3	13.8	0.47	340	71.75	27.75	4.32.8
Av.	13.8	13.15	0.46	—	70.73	29.20	—
Standard deviation	—	—	—	—	0.84	0.85	—

4° WHEATS							
Wt/bu ¹	Wheat protein	Flour protein	Ash	GP ²	Total ³ flour	Total feed	Yield
61½	13.8	12.7	0.49	518	69.30	30.50	4.43.0
60	13.9	12.9	0.49	576	67.25	32.30	4.51.8
60½	14.2	13.5	0.49	416	69.70	30.25	4.41.0
60	14.3	13.8	0.49	438	70.10	29.85	4.40.0
60	13.7	12.9	0.49	490	68.70	31.40	4.45.0
60½	13.3	12.6	0.48	610	67.00	33.30	4.52.8
60½	13.6	12.7	0.50	440	69.10	31.35	4.43.8
60	13.7	12.8	0.48	410	67.39	33.05	4.50.12
60	13.5	12.5	0.48	528	67.10	33.25	4.52.0
60	13.6	12.7	0.48	464	69.50	30.15	4.42.0
Av.	13.75	12.9	0.487	—	68.51	31.54	—
Standard deviation	—	—	—	—	1.20	1.35	—

¹ Wt/bu = weight per bushel—Imperial measure.

² GP = gasping power (Sandstedt and Blish, Cereal Chem. 11: 381-2, 6 hours, 30°C).

³ Total flour = computed on basis of total products.

and securing duplicate results, three samples of replicate millings were made. The first, second, and third samples contained five replicate millings each of 2°, 3°, and 4° wheats respectively. The flour yields of these millings were reduced by means of statistical analysis. The method of analysis of variance is described by Goulden.²

The error within the samples was found to be not significant and indicates that milling results can be duplicated on the Buhler. The variation between the grades is large and shows the effect of the physical character of the wheat upon the flour yield.

The accuracy of mill control which bears some relation to the skill and care of the operator is indicated in the standard deviation of the shorts and bran. It will be noted that the error of replication is not necessarily higher among the lower grades of wheat. Duplication of milling results can therefore be obtained with sufficient accuracy in any grade of wheat.

TABLE VII
SUMMARY OF MILLING RESULTS OF VARIOUS STATUTORY GRADES

Grade	Wt/bu	Yield	Yield	Ash	Break flour	Feed	No. samples
		%	bu/bbl	%	%	%	
1°	63½	73.48	4.27.0	0.452	16.86	26.75	7
2°	62½	73.15	4.28.0	0.464	17.30	26.70	50
3°	62	71.59	4.33.12	0.465	17.00	28.30	19
4°	61	69.08	4.43.12	0.480	15.70	30.80	18
Garnet	64½	71.46	4.34.8	0.493	13.75	28.00	16
Special	56	68.89	4.47.0	0.510	16.70	30.90	10

In Table VI, are shown milling results of 2°, 3°, and 4° wheats respectively. These results were taken at random from among numerous millings of wheat shipments during a single crop year. The variation within the samples increased with each decrease in grade. The tendency of the physical character of the lower grades of wheat to vary more widely indicates that wheats of similar statutory grade may be classified further according to their respective milling values. As far as the statutory grades are concerned, it is usually the case that wheats of poorest milling value possess the poorest baking quality.

Table VII shows a summary of the milling results of various grades of wheats. They indicate that the flour yield is influenced by the physical condition of the wheat rather than by the weight per bushel.

Correlation of the Buhler and Commercial Milling Results

The analysis of the Buhler milling results in Table V indicated that the error of replication was not significant. A survey of a series of commercial milling results indicated that the error of replication

² C. H. Goulden: *Methods of statistical analysis*, John Wiley and Sons, New York, 1939.

was smaller than that of the experimental mill. It was concluded, therefore, that a correlation with respect to flour yield should exist between the Buhler and commercial units. As a preliminary experiment, it was arranged to mill a sample blend of wheat on the commercial unit for six consecutive days. Samples of the wheat blend and straight-grade flour were taken daily and the flour yields (total products) for each day were calculated. The six samples of the wheat

TABLE VIII
COMPARATIVE ANALYSES OF COMMERCIAL AND BUHLER FLOURS

Wheat protein	Flour protein	Ash	GP ²	Flour ¹	Feed	Total products	Yield ³ as found	Moisture as rec'd	Yield
%	%	%		%	%	%	%	%	bu/bbl
BUHLER									
14.2	13.3	0.46	460	73.6	26.00	98.50	76.23	10.4	4.26.5
14.1	13.3	0.46	460	73.4	26.05	98.25	75.85	10.6	4.27.0
14.2	13.4	0.48	480	72.0	27.60	98.70	74.16	10.9	4.33.9
14.2	13.3	0.48	450	72.0	27.40	98.20	74.41	10.6	4.33.9
14.2	13.3	0.47	492	73.0	26.55	98.30	75.44	10.6	4.28.8
14.2	13.4	0.48	510	72.0	27.50	98.20	74.50	10.5	4.33.9
Av. 14.2	13.3	0.47	475	72.67	26.85	98.36	75.10	10.6	4.29.11
COMMERCIAL									
14.2	13.5	0.46	500	73.2	26.8	100.0	74.40	12.4	4.27.11
14.2	13.5	0.45	500	73.6	26.4	100.0	74.49	12.4	4.26.5
14.2	13.5	0.46	460	73.3	26.7	100.0	74.20	12.5	4.27.5
14.2	13.6	0.46	496	73.2	26.8	100.0	74.50	12.0	4.27.11
14.2	13.6	0.46	520	73.15	26.85	100.0	74.12	12.4	4.27.15
14.2	13.7	0.46	520	72.85	27.15	100.0	73.75	12.6	4.29.0
Av. 14.2	13.55	0.46	499	73.21	26.79	100.0	74.18	12.4	4.27.11

¹ Total flour computed on basis of total products.

² GP—gassing power (Sandstedt and Blish, Cereal Chem. 11: 381-382, 6 hours, 30°C).

³ Yield—"as found" moisture basis.

blend were then conditioned according to experimental method and milled on the Buhler. The data covering the six days' operation of the commercial unit and the corresponding results of the Buhler millings are shown in Table VIII. The yield results were reduced by statistical analysis. The theory involved in the determination of the correlation coefficient and the test of significance of the coefficient is described by Goulden.

$$\begin{array}{rcl}
 \Sigma(x^2) & = & 45.52 \\
 T_x^2/6 & = & 42.67 \\
 \hline
 \Sigma(x - \bar{x})^2 & = & 2.85 \\
 \Sigma(xy) & = & 52.18 \\
 T_x T_y / 6 & = & 51.467 \\
 \hline
 \Sigma(x - \bar{x})(y - \bar{y}) & = & .713
 \end{array}
 \qquad
 \begin{array}{rcl}
 \Sigma(y^2) & = & 62.375 \\
 T_y^2/6 & = & 62.0817 \\
 \hline
 \Sigma(y - \bar{y})^2 & = & .2933 \\
 \hline
 r_{xy} & = & \frac{.713}{2.85 \times .2933} = .854
 \end{array}$$

To determine the significance of r_{xy}

$$t = \frac{r\sqrt{n}}{\sqrt{1-r^2}} = 3.28 \quad n = 4 \text{ (degrees of freedom)}$$

x = Buhler yield

y = commercial yield

Since t at the 5% level of probability equals 2.78, the correlation is statistically significant.

The covariation of the Buhler and commercial flour yields is shown in the regression graph, Figure 2. The regression equation is $Y = 54.54 + .257x$, where Y equals the estimated values of y .

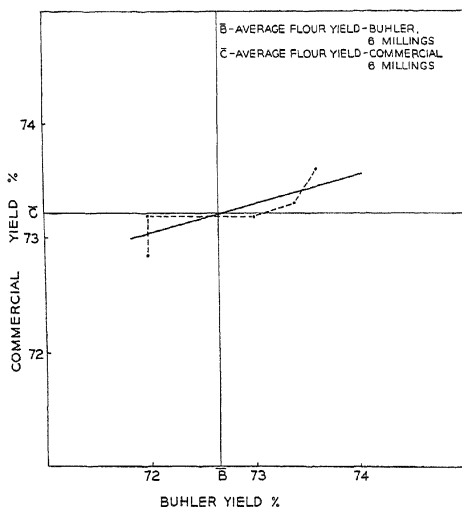


Fig 2 Regression graph of comparative flour yields.

The above series of milling tests was repeated and although there was a slight increase in error of replication in the Buhler results, the correlation was still significant.

It appears probable that experimental error can be minimized sufficiently by the proper technique and care of the operator so that a significant correlation would exist.

A Comparison of the Buhler and Commercial Flours

The respective straight-grade flours were accepted as a basis for comparison, since it was found that certain characteristics are apparent in the break flour and are peculiar to different grades of wheat.

The standard A. A. C. C. baking formula was used but with 200 g of flour (13.5% moisture basis), also a bromate (0.001%) and fermentation differential (2 hours, 40 minutes; 3 hours, 40 minutes). In the initial test, four loaves were baked from each flour. A modification included the addition of a malt supplement depending on the gassing power of the experimental flour. Other supplementary bakings included modifications of the fermentation time, depending on the protein content of the flour. The flours milled during the six days' operation of the commercial unit and the corresponding flours milled on the Buhler were analyzed and baked. The protein conversion

TABLE IX
COMPARATIVE LOAF VOLUMES OF BUHLER AND COMMERCIAL FLOURS

Fermentation time	Buhler volume		Commercial volume	
	Initial ¹	Stimulated ²	Initial ¹	Stimulated ²
2 hours, 40 minutes	cc	cc	cc	cc
	690	720	700	725
	695	715	710	730
	710	715	695	720
	680	725	715	725
	690	710	705	720
	710	730	710	720
Average	696	720	706	723
3 hours, 40 minutes	660	685	685	685
	630	680	670	665
	630	700	665	670
	645	685	675	685
	650	700	675	685
	650	680	660	680
Average	644	688	671	678

¹ Initial: Standard A. A. C. C. formula

² Stimulated: A. A. C. C. formula plus 10 ppm KBrO₃ (0.001%)

(difference between wheat and flour protein) of the two flours was similar and the ash contents compared favorably. Approximately the same flour extraction, therefore, was obtained in each case.

The flours were stored in a fermentation cabinet until they attained a moisture content of 13.5%. The absorption was then determined by mixing a few trial doughs. The same absorption was used for each flour and a dough of similar consistency was obtained in each case. Dough handling properties seemed much the same throughout the samples. The loaf volumes are given in Table IX. A summary of the baking analyses indicated a definite similarity with respect to loaf volume, crust, and crumb character.

Flour Color

Although we have not determined the respective carotene contents, the color of the Buhler flour appears somewhat more yellow than that of the commercial, but color comparisons among experimental flours are readily made.

Granulation

The Buhler flour is more granular to the feel than the commercial flour even though the actual granulation is similar, but this condition does not appear to cause any wide discrepancies in either the baking test or the gassing power. The gassing power of the commercial flour may be reliably predicted on the basis of experimental results.

Summary of Milling and Baking Value

Our results of an experimental milling and baking test are summarized as to milling and baking value. The milling value includes the classification of the wheat with respect to the physical condition of the kernels, the observations recorded of the milling behavior, and the flour yield. The observations of the milling behavior of the grain are of course limited, due to the short system and the lack of classification of stocks. The baking value is primarily an interpretation of the "blending value" and "carrying capacity" of the wheat with respect to its value in a commercial blend.

Milling Technique

A number of points are considered important with respect to milling technique and may be enumerated as follows:

1. A preliminary warm-up of the mill is considered necessary since adjustments of roll settings were made when the mill was warm.
2. The feed gate above second reduction roll should be adjusted to back up the stock sufficiently to maintain an even and steady rate of feed to the roll. This has much to do with having the proper load on the tail of the mill.
3. Since the mill does not carry a full load at the start of a run, the abrasive action on the stocks tends to throw the flour off color. At the end of the run the same action occurs, and in addition when the mill is swept out, any accumulation of foreign material is swept into the flour, with the result that the flour is dark in color and high in ash. Portions of flour are therefore taken off at the first and last of a run, weighed up and added into the gross weight in computing the yield. The flour produced during the middle of the run is used for color, chemical, and baking tests.

4. It was noticed that regardless of how well the mill was swept out, after a number of millings there was an accumulation of foreign material within the mill which appeared to get into the flour occasionally and affect the color. It is considered wise therefore to thoroughly "blow out" the mill with compressed air after each day's milling.

Relative Humidity

Humidity conditions in our mill room vary between 50% and 90% in winter and summer. These extreme conditions appear to have an effect on our milling results. Higher humidity tends to decrease the capacity and bolting efficiency of the mill. Although we have been unable to experiment in this connection, it would appear that milling conditions in an air-conditioned mill room would be much better controlled.

Summary

Although the personal factor is evident, the duplication of milling results with the Buhler is possible under controlled conditions and technique. Little adjustment of the primary mill set-up is required when grinding wheats of varying weight per bushel, apart from regulation of rate of feed.

The error of replication in both the experimental and commercial millings was found not to be significant. The flour characteristics and baking values of the Buhler and commercial flours were sufficiently comparable for reliable interpretation. It is concluded then that the Buhler mill is useful for predetermining commercial milling and baking results.

A CRYSTALLINE PROTEIN OBTAINED FROM A LIPOPROTEIN OF WHEAT FLOUR

A. K. BALLS, W. S. HALE, and T. H. HARRIS

Enzyme Research Laboratory,¹ Bureau of Agricultural Chemistry and Engineering,
U. S. Department of Agriculture, Washington, D. C.

(Received for publication October 9, 1941)

The occurrence of a curious, hitherto unsuspected sulphydryl compound in wheat flour was reported recently from this laboratory by Balls and Hale (1940). The substance, apparently in combination with a lecithin-like lipid, was extracted from flour by petroleum ether. The extract, after some purification, was hydrolyzed with hydrochloric acid and then yielded a sulfur-containing material which no longer resembled a lipid, but behaved like a protein. This material was

¹ Enzyme Research Laboratory Contribution No. 71.

soluble in water and dilute alcohol, but not in fat solvents; from aqueous solutions it could be salted out by ammonium sulfate; it contained roughly 13% of nitrogen and no measurable amount of phosphorus. Nearly 3% of the material was sulfur, no longer in the $-SH$ form, but present as cystine.

Further investigation has shown that such material is not a single substance. Considerable variation in the content of nitrogen and sulfur has been encountered between different preparations. One of the substances present was, however, found to remain in solution in 70% ethyl alcohol, but to precipitate when the concentration of alcohol was increased to about 90%. On repetition of this treatment, the precipitate was crystalline. The crystals formed rosettes of needles, somewhat reminiscent of tyrosine. Two subsequent recrystallizations did not change the apparent crystal form or cause a significant change in the content of sulfur or nitrogen. One constituent of the original materials is thus obtainable in reproducible form. The purpose of this paper is to report observations made in a chemical study of this crystalline substance.

Method of Preparation

The following example will serve to illustrate the method of preparation:

200 pounds of freshly milled unbleached patent flour from soft wheat was extracted with high-grade petroleum ether in a percolator. Nearly all the solvent was removed from the extract by distillation in vacuum; the liquid extract was kept at $-1.5^{\circ}C$ for several weeks and then separated in a refrigerated centrifuge from crystals of sterol that had deposited during cold storage. The liquid was next mixed with an equal volume of ether and then with three volumes of cold 1*N* hydrochloric acid in absolute ethyl alcohol. The mixture stood at 0° for an hour; then the precipitate that formed was separated in a centrifuge and thoroughly washed, first with absolute alcohol and then with ether. After drying, 25.2 g of material comparable to that described in our previous paper was obtained.

This crude material, dissolved in 100 ml of water, was mixed with 300 ml of absolute alcohol. The precipitate that formed was removed in the centrifuge, and the supernatant liquid was evaporated somewhat on the steam bath and finally to dryness in vacuum over P_2O_5 . The dry residue weighed 16.8 g.

15.5 g of this residue was dissolved in 25 ml of water; 225 ml of absolute alcohol was then added and the mixture was allowed to stand at 5° for 4 hours. An entirely crystalline precipitate formed, whose weight after drying was 4.10 g. The crystals are shown in Figure 1.

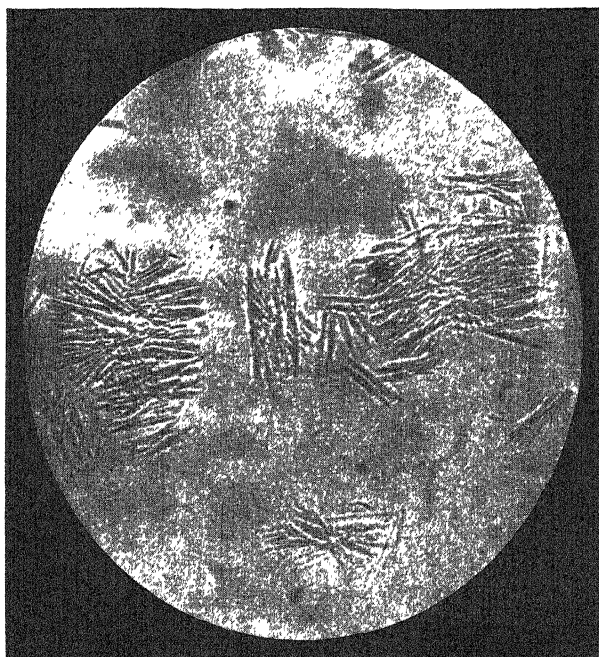


Fig. 1 Protein obtained from a lipoprotein of wheat flour

Recrystallization was made in the same manner as the step just described. For the analytical work reported here thrice crystallized material was used. Its composition was essentially the same as that of material twice crystallized, as shown by the data in Table I.

TABLE I
COMPOSITION AFTER RECRYSTALLIZATION

	Nitrogen	Chlorine	Sulfur
	%	%	%
Crystallized once	16.20	6.64	4.49
			4.31
Crystallized twice	17.25	6.56	4.46
	17.50	6.57	
Crystallized thrice	17.45	—	—
	17.10		

Analytical Results

The crystalline material was found to be very soluble in water, and in dilute alcohol. The water solution gave a positive biuret and a

positive Molisch test.² Hydrolysis in constant-boiling HCl (120°) for six hours produced nearly an eightfold increase in amino nitrogen.³ The amino nitrogen after hydrolysis was also about three-fourths of the total nitrogen originally present. The data appear in Table II. Hydrolysis for 18 hours instead of 6 caused no significant change in the results.

TABLE II
AMINO NITROGEN BY VAN SLYKE METHOD

	Sample	Amino nitrogen
	mg	%
Original material	4.75	1.65
	4.75	1.65
After acid hydrolysis ¹		Av. 1.65
	2.63	11.99
	2.63	13.03
	5.25	12.50
	5.25	12.60
		Av. 12.53

¹ Boiled 6 hours with 20% HCl at 120°; samples from a stock solution of the hydrolysate, represented 2.63 mg original substance per milliliter.

A very small droplet of heavy, oily liquid always remained after hydrolysis. The amount of this "oil" was not great enough to affect the analytical results appreciably. The "oil" did not appear to diminish when the period of hydrolysis was extended from 6 to 18 hours, and the droplet was insoluble in strong, hot solutions of either HCl or NaOH.

Determinations of individual constituents were made by the methods and with the results shown in Table III. On the basis of these determinations the equivalent proportions were computed, and are shown in Table IV. Ammonia was absent from the hydrolysate, indicating the absence of acid amide nitrogen in the original.

Probable Molecular Weight

A minimum value for the molecular weight of the substance may be calculated from the data of Table IV. Assuming the presence of one tyrosine residue in the molecule, this minimum is approximately 6,000.

With such molecules, it is customary to attempt an estimation of the size by determination of the rate of diffusion. To this end the technique of Northrop and Anson (1928-29) was used. The diffusion cell was calibrated with 2*M* NaCl.

² A test with the orcin reagent of M. Sørensen indicated that the amount of carbohydrate present was less than 0.5%.

³ Determined in the volumetric apparatus of Van Slyke after 3 minutes' shaking with the customary nitrous acid reagent. Shaking for 10 minutes gave the same result.

A solution of 3.33 mg per ml in 0.5*M* NaCl was allowed to diffuse for 26 hours against an equal volume of 0.5*M* NaCl. Nitrogen determinations gave a diffusion coefficient of 0.155 cm²/day, a molecular radius of 1.44×10^{-7} cm, and thus a molecular weight (assumed density = 1.3) of 10,200. This value is an indication that under the conditions described, the molecular weight is about 12,000, namely twice that calculated from the analyses.

TABLE III
ANALYSIS OF THRICE-CRYSTALLIZED MATERIAL

Component sought	Method used	Sample taken	Amount found
		mg	%
Chlorine	Pregl (1937)	22.860	6.57
		20.215	6.56
Sulfur	Pregl (1937a)	23.900	4.49
		23.500	4.31
		21.789	4.46
Nitrogen	Kjeldahl after Pregl (1937b)	4.409	17.22
		3.279	17.32
		2.797	17.45
Phosphorus	Fiske and Subbarow (1925)	2.00	None ¹
Selenium	A. O. A. C. (1940)	50.0	None ²
Ash	Residue from a chlorine determination	11.036	0.06
Arginine	Thomas, Ingalls and Luck (1939)	12.68	20.4
Cystine	Sullivan and Hess (1930)	11.43	15.7
Tyrosine	Bernhart (1938)	16.79	3.0
Tryptophane	Block (1938)	16.79	Trace ³

¹ Therefore less than 0.05% phosphorus.

² The method is said to detect 3×10^{-7} mg of selenium. The authors wish to thank Mr. Hubert W. Lakin of the Bureau of Plant Industry for making the test.

³ The tryptophane determination was carried out on the HgSO₄ precipitate from the tyrosine determination. The color value corresponded to 0.3% tryptophane, but was probably due to a trace of tyrosine. The value obtained for the molecular weight makes it probable that the finding of any amino acid in so small an amount is an error.

TABLE IV
CONSTITUTION OF THRICE-CRYSTALLIZED SUBSTANCE

Constituent	Percent of weight	Percent of total N	Proportion of equivalents ¹
Chlorine	6.57	—	11.0
Sulfur	4.42	—	8.2
Nitrogen	17.35	(100)	73.8
Amino nitrogen by Van Slyke method	1.65	9.5	7.0
Arginine	20.4	37.8	6.9
Cystine	15.7 ²	10.5	3.9
Tyrosine	3.0	1.3	1.00
Unaccounted for ³	—	40	—

¹ Based on tyrosine = 1.00.

² Equivalent to 4.18% sulfur, or 95% of the total sulfur found. It is thus probable, though not proved, that all the sulfur exists as cystine.

³ The assumption is made that the Van Slyke amino nitrogen is not derived from arginine. If the difference between original total nitrogen and amino nitrogen after hydrolysis (see Table II) is regarded as due entirely to proline, 28% of the total nitrogen would be proline, leaving only 12% unaccounted for. The several serious errors probably inherent in such speculation all tend to make the value for the supposed proline too high, nevertheless, proline or a related group may well be present in a very considerable amount.

A molecule of this size could scarcely be expected to give a measurable sedimentation constant in our ultracentrifuge. In an experiment in which a solution of crystals in water containing 3.1 mg per ml was centrifuged for 3 hours at a mean centrifugal force of 91,000 \times gravity, no sedimentation was in fact observed.

Behavior in the ultracentrifuge thus appears to agree with the diffusion measurement, but it must be admitted that both methods require unwarranted assumptions with regard to the shape of the particles. The computations have here been based on spherical particles.

Remarks on Constitution

It may be argued with fair success that the only completely inclusive description of a protein is a substance that yields amino acids on hydrolysis. The production of amino acids by hydrolysis of this crystalline material from wheat shows that a good part of the molecule has the composition ascribed to proteins or peptides. Well over half the total nitrogen has been definitely accounted for as arginine, cystine, and tyrosine. Some of the remainder is probably proline or a related substance; but the existence of a nitrogen-containing group which is not an amino acid has not been excluded.

A formal distinction between protein and nonprotein nitrogen is often based on behavior toward trichloroacetic acid. The usual method of separating protein from nonprotein nitrogen is to precipitate the former from solution by the addition of trichloroacetic acid to a concentration of 0.15*M*. The mixture is then held briefly at 70°, and the precipitated protein is finally separated. Under such conditions a half-percent solution of the crystals in question gave no precipitate, but when the concentration of trichloroacetic acid was increased to 0.4*M*, 97% of the nitrogen was precipitated. The precipitate, however, completely redissolved on warming, and reappeared on cooling.

It is evident that the substance lies on the borderline between true proteins and bodies of smaller molecular dimensions. One point of resemblance to proteins is the ease with which solutions undergo some change akin to polymerization or denaturation, whereby the substance becomes no longer readily soluble in water or dilute acid. Such a change occurs when a solution in water is electrolyzed. The substance naturally gives positive results with the biuret, xantho-proteic, and Millon tests. It will probably be more convenient to refer to the substance as a protein, if the word is not permitted to imply too much.³

The crystals are evidently a hydrochloride. Silver chloride may be precipitated directly by adding silver nitrate to an aqueous solution.

³ The authors will appreciate any carefully made suggestion of a name for this substance.

The acidity of such aqueous solutions is furthermore very high. A solution of 1 mg per ml in water was found to be at pH 3.85. This value approximates a 5% dissociation of all the chlorine (if no other acid groups interfered). The base is therefore a strong one, in spite of its high cystine content.

Substances resembling our material from wheat in molecular size and general make-up are well known, but examples are not very common. Among such are the protamines, the inhibitors of trypsin and of pepsin, and the bactericidal substances recently described by Dubos and Cattaneo (1939).

The protamines, like our material, are strongly basic and contain much arginine. We are not aware, however, of any recognized protamine that contains cystine. Trypsin inhibitor, crystallized and studied by Kunitz and Northrop (1936), has the general properties of a polypeptide with a molecular weight of about 6,000. The inhibitor of pepsin, crystallized and studied by Herriott (1941), also contains amino acids and resembles our material in size (mol wt 4,000-10,000) and in high content of arginine. Two crystalline substances, gramicidin and tyrocidin, isolated from a soil microorganism by Dubos and Cattaneo (1939) and Hotchkiss and Dubos (1940), are composed largely of amino acids. They appear to be smaller molecules than the substance with which we have worked. Gramicidin is soluble in acetone and in a mixture of acetone and ether, and has a molecular weight of 1400. Tyrocidin is soluble in hot absolute alcohol. Sulfur has not been reported as a constituent of either body, but tyrocidin forms a crystalline hydrochloride. There is a point of resemblance between these substances and that isolated from wheat in that the latter is also a very powerful bactericide. This property will be reported in detail in a later paper.

The material isolated from wheat is thus quite distinct from the somewhat similar substances just discussed. In the grain, it undoubtedly exists in a reduced form containing $-SH$ groups. There can be little question that such an $-SH$ compound in wheat and flour increases the activity of the papain-like enzyme present in both. The substance is apparently a natural activator of the wheat proteinase.

The original solubility of the substance in petroleum ether shows that it occurs in combination with a lipid. Such solubility is not a mixed-solvent effect. The mere presence of flour lipids in solution in gasoline or ether was not sufficient to redissolve the protein-like body, once that had been prepared in the water-soluble state. Nor is the solubility in petroleum ether due to the original reduced condition of the substance, for it remains soluble after the $-SH$ groups have been oxidized by air. Thereafter it may be precipitated, along with some

lipoidal material, by ethyl acetate or by alcohol. The precipitate made with acetate is easily soluble again in petroleum ether or in ether, and may be precipitated and redissolved repeatedly.

The ethyl acetate precipitate was found to contain about 1% of phosphorus. It was only partly soluble in hot absolute alcohol and the insoluble fraction had roughly the same phosphorus content as the original. Continued treatment with hot neutral alcohol slowly removed the phosphorus from the insoluble fraction, but the addition of hydrochloric acid to the alcohol caused rapid removal, even in the cold. An experiment is detailed in Table V. It should be remembered that this ethyl acetate precipitate contains other protein-like substances, apparently similar to, but not identical with, the one that was crystallized as is reported here.

TABLE V
BEHAVIOR OF AN ETHYL ACETATE PRECIPITATE

Treatment	Wt of fraction	Composition	
		N	P
	mg	%	%
(1) Original ¹	1,000	4.21	1.15
(2) One gram of (1) heated to 78° in 25 ml of absolute alcohol, centrifuged at 60°-70°. Sediment only	110	8.58	0.99
(3) Sediment (2) resuspended 25 ml of boiling alcohol for 15 min and recentrifuged. Sediment only	—	10.4	—
(4) Sediment (3) boiled 30 min in 25 ml of absolute alcohol, centrifuged. Sediment only	—	10.4	0.57
(5) Part of sediment (4) suspended in absolute HCl alcohol (1 <i>N</i>) at 50° for 15 min. Centrifuged, sediment only	—	11.7	0.16
(6) 95 mg of sediment (4) suspended in 2 ml 1 <i>N</i> HCl alcohol at 0° and kept at 0° for 2 days. Sediment washed well with dry ether	59	10.8	0.28

¹ Precipitated by adding 3 volumes of ethyl acetate to the liquid portion of the flour lipoids as originally extracted by petroleum ether. Precipitate washed 3 times (centrifuge) with 1 volume of cold ethyl acetate.

It is unlikely that a free lipid like lecithin can act in this manner toward ethyl acetate, hot alcohol, and cold acid-alcohol. In any case, repeated treatments with large volumes of boiling alcohol should remove lecithin. A lecithin-like lipid in combination with a protein, however, might behave in the manner observed.

In view of the basic nature of our substance, a reasonable working hypothesis seems to be that it is either associated with or replaces choline or other base in a lecithin-like lipid. Whatever the form of connection between protein-like fraction and lipid may be, it seems evident that the mother substance of our crystalline product belongs

in the vaguely defined class of lipoproteins. It is thought that a beginning has been made in the description of one fragment of a lipoprotein.

Summary

A crystalline substance of protein-like nature has been obtained from the petroleum ether extract of wheat flour. Analytical examination has shown that the substance consists mainly of amino acid residues. Arginine, cystine, and tyrosine account for over half of the total nitrogen. The crystals are a hydrochloride of a basic substance. This hydrochloride has a minimum molecular weight of 6,000, and a probable molecular weight of double that value. The substance therefore lies in the borderline between proteins and polypeptides, where such protein tests as precipitation with trichloroacetic acid are indefinite. In the grain and in flour the material exists in the reduced form as a sulphydryl compound. It is probably a natural activator of the wheat proteinase. The method of isolation and the behavior of the substance during purification lead to the belief that the protein-like substance exists in flour combined with a phosphorus-bearing lipid, and is therefore a component of one member of the little understood class of lipoproteins.

Literature Cited

- Association of Official Agricultural Chemists
 1940 Methods of analysis (5th ed.), p. 417. Washington, D. C.
 Balls, A. K., and Hale, W. S.
 1940 A sulfur-bearing constituent of the petroleum ether extract of wheat flour (preliminary report). *Cereal Chem.* 17: 243-245.
 Bernhart, F. W.
 1938 Microdetermination of the tyrosine content of protein hydrolysates. *Proceedings 32nd Meeting A. S. B. C., J. Biol. Chem.* 123: x.
 Block, R. J.
 1938 Determination of amino acids (rev. ed.), p. 22. Burgess Publishing Co., Minneapolis, Minnesota.
 Dubos, R. J., and Cattaneo, C.
 1939 Studies on a bactericidal agent extracted from a soil bacillus. III. Preparation and activity of a protein fraction. *J. Exp. Med.* 70: 249-256.
 Fiske, C. H., and Subbarow, Y.
 1925 The colorimetric determination of phosphorous. *J. Biol. Chem.* 66: 375-400.
 Herriott, R. M.
 1941 Isolation, crystallization, and properties of pepsin inhibitor. *J. Gen. Physiol.* 24: 325-338.
 Hotchkiss, R. D., and Dubos, R. J.
 1940 Bactericidal fractions from an aerobic sporulating bacillus. *J. Biol. Chem.* 136: 803-804.
 Kunitz, M., and Northrup, J. H.
 1936 Isolation from beef pancreas of crystalline trypsinogen, trypsin, a trypsin inhibitor, and an inhibitor-trypsin compound. *J. Gen. Physiol.* 19: 991-1007.
 Northrup, J. H., and Anson, M. L.
 1928-29 A method for the determination of diffusion constants and the calculation of the radius and weight of the hemoglobin molecule. *J. Gen. Physiol.* 12: 543-554.

- Hregl, F.
1937 Quant. organic microanalysis (3rd Eng. ed.), p. 86. P. Blakiston's Son and Co., Philadelphia.
1937a Quant. organic microanalysis (3rd Eng. ed.), p. 94. P. Blakiston's Son and Co., Philadelphia.
1937b Quant. organic microanalysis (3rd Eng. ed.), p. 116. P. Blakiston's Son and Co., Philadelphia.
Sullivan, M. X., and Hess, W. C.
1929 Studies on the biochemistry of sulfur. IV. Supplement No. 78, U. S. Public Health Reports.
Thomas, L. E., Ingalls, J. K., and Luck, J. M.
1939 The determination of arginine in the presence of other amino acids by means of the Sakaguchi reaction. J. Biol. Chem. 129: 263-271.

BACTERICIDAL AND FUNGICIDAL PROPERTIES OF A CRYSTALLINE PROTEIN ISOLATED FROM UNBLEACHED WHEAT FLOUR

L. S. STUART¹ and T. H. HARRIS²

Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture,
Washington, D. C.

(Received for publication October 9, 1941)

The discovery of "in vitro" and "in vivo" bactericidal activity for certain crystalline polypeptides by Dubos (1940), Dubos and Hotchkiss (1941), and Dubos and Cattaneo (1939) suggested the study of a somewhat similar crystalline protein recently separated from the petroleum ether extract of unbleached wheat flour for similar properties. Balls, Hale, and Harris (1942) separated and gave a rather complete chemical description of this protein. It differs from the alcohol-soluble, water-insoluble polypeptides designated as "gramicidin" and "tyrocidin" by Hotchkiss and Dubos (1940) and Dubos and Hotchkiss (1941) in that it is water-soluble, alcohol-insoluble, contains about 4.5% sulfur in the form of cystine, and is completely precipitated by 2.5% trichloroacetic acid.

It appears to be the oxidized form of a powerful oxidation-reduction system and, therefore, might be expected to have considerable biological activity.

"In Vitro" Bactericidal Tests

In the "in vitro" testing for bactericidal activity the selection of a suitable method is always a "moot" question. Results by any single method may fail to give a complete picture of the limits of activity. For this reason three divergent methods were employed in these studies. These methods may be designated as: (1) the broth culture

¹ Associate Bacteriologist, Hides, Tanning Materials, and Leather Division, Eastern Regional Research Laboratory, Wyndmoor, Pennsylvania.

² Assistant Chemist, Enzyme Research Laboratory, Washington, D. C.

TABLE I

EFFECT OF CRYSTALLINE PROTEIN ON BACTERIA IN BEEF-HEART INFUSION AND GLUCOSE BROTH AFTER INCUBATION AT 37.5°C FOR 48 HOURS

Test Organism								
Protein added as the hydrochloride	<i>Streptococcus viridans</i>		<i>Pneumococcus Type I</i>		<i>Pneumococcus Type III</i>		<i>Sarcina lutea</i>	
	Bacterio- stasis	Via- bility	Bacterio- stasis	Via- bility	Bacterio- stasis	Via- bility	Bacterio- stasis	Via- bility
<i>mg per ml</i>								
0.5	—	+	—	+	—	+	—	—
0.25	—	+	—	+	—	+	—	—
0.10	—	+	—	+	—	+	—	—
0.05	—	+	—	+	—	+	—	—
0.025	—	+	—	+	—	+	—	+
0.001	—	+	+	+	+	+	—	+
0.0005	+	+	+	+	+	+	+	+
0.00	+	+	+	+	+	+	+	+

Test Organism								
Protein added as the hydrochloride	<i>Staphylococcus aureus</i>		<i>Escherichia coli</i>		<i>Eberthella typhi</i>		<i>Pseudomonas pyocyaneus</i>	
	Bacterio- stasis	Via- bility	Bacterio- stasis	Via- bility	Bacterio- stasis	Via- bility	Bacterio- stasis	Via- bility
<i>mg per ml</i>								
0.5	—	+	+	+	+	+	+	+
0.25	—	+	+	+	+	+	+	+
0.10	—	+	+	+	+	+	+	+
0.05	—	+	+	+	+	+	+	+
0.025	+	+	+	+	+	+	+	+
0.001	+	+	+	+	+	+	+	+
0.0005	+	+	+	+	+	+	+	+
0.00	+	+	+	+	+	+	+	+

+ = growth.

— = no growth.

method of Dubos and Cattaneo (1939); (2) the agar-cup plate method of Reddish (1929); and (3) the accelerated death curve method of Cade and Halvorson (1934).

With the broth culture method *Streptococcus viridans*, *Pneumococcus Type I*, *Pneumococcus Type III*, *Staphylococcus aureus*, *Sarcina lutea*, *Eberthella typhi*, *Escherichia coli*, and *Pseudomonas pyocyaneus* were employed as test organisms. The crystalline protein hydrochloride was added to tubes of nutrient beef-heart infusion and glucose broth³ after sterilization. The amounts added were equivalent to 0.5, 0.25, 0.1, 0.05, 0.025, 0.001, and 0.0005 mg per ml of broth. Control tubes of broth without added protein hydrochloride and tubes con-

³ 1,000 ml beef-heart extract and 1,000 ml distilled water containing 10 g NaCl, 0.4 g MgSO₄, 2.0 g K₂HPO₄, 0.2 g CaCl₂, 20 g bacto-peptone, and 2.0 g of glucose, adjusted with NaOH to pH 7.5; sterilized at 15 lbs for 20 minutes.

taining the amounts specified above were each inoculated with one loop of inoculum and incubated at 37.5°C for 48 hours. The loop inocula were taken from 48-hour cultures of the test organisms in Brewer's (1940) semisolid thioglycolate broth.

After 24 and 48 hours of incubation all broth cultures were examined for visible signs of growth. The absence of visual signs of growth was interpreted as evidence of bacteriostasis. After 48 hours all test cultures were subcultured, using a loop needle transfer back into thioglycolate broth. These subcultures were in turn incubated for 48 hours at 37.5°C. Absence of growth in subcultures was interpreted as evidence of bactericidal activity. The results of tests using this method are recorded in Table I.

As shown in Table I only one of the test organisms was completely killed—namely, *Sarcina lutea*. However, all of the Gram-positive organisms were definitely inhibited with the higher concentrations of the protein. The Gram-negative organisms *Escherichia coli*, *Eberthella typhi*, and *Pseudomonas pyocyaneus* were neither inhibited nor killed. Thus, based on this method, the protein would appear to have slight bactericidal activity and strong bacteriostatic action only so far as Gram-positive organisms are concerned.

With the agar-cup plate method, the agar medium used was made up at pH 6.9 according to the latest directions given by Ruhle and Brewer (1931). Only two test organisms were employed—*Staphylococcus aureus* and *Eberthella typhi*. In each case six tubes each containing 15 ml of sterile melted agar, were cooled to 45°C, after which they were inoculated with 0.1 ml of a 24-hour plain broth culture. An individual plate was poured from each tube and the agar was allowed to solidify at room temperature. Disks were then reamed out of the solidified agar by means of a sterile metal cork borer, leaving cups. Into these cups 0.5-ml portions of the solutions to be tested were placed. Water solutions containing 1.0, 0.5, 0.25, 0.10 and 0.05 mg of the crystalline protein hydrochloride were used. All plates were incubated for 24

TABLE II
RESULTS OF AGAR-CUP PLATE TESTS FOR BACTERICIDAL ACTIVITY

Amount of protein hydrochloride in cup	Test organism	
	<i>Staphylococcus aureus</i> inhibition zone	<i>Eberthella typhi</i> inhibition zone
mg per ml		
0.500	present	present
0.250	"	"
0.120	"	"
0.050	"	"
0.025	"	absent
0.000	absent	"

hours at 37.5°C under unglazed porcelain tops to prevent the condensation of water of syneresis. The results of these tests are given in Table II. Figure 1 illustrates clearly the types of zones of inhibition obtained around the agar-cups with each test organism as indicated in Table II. From Table II it can be seen that zones were formed with the Gram-positive *Staphylococcus aureus* with all concentrations of the protein tested, whereas with the Gram-negative *Eberthella typhi* no zone was formed with 0.5 ml of solution containing 0.025 mg per ml of the protein. In no instance did inhibition appear to be complete.

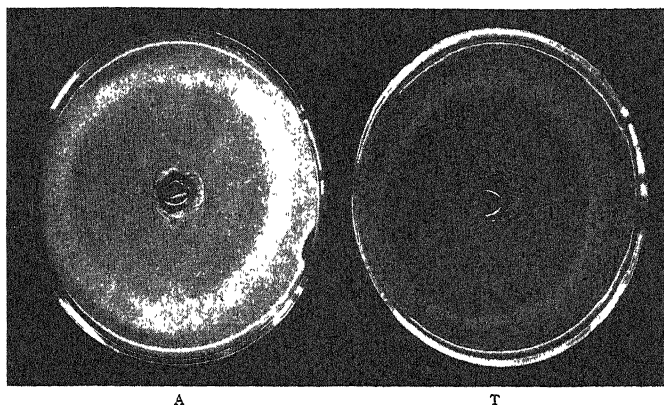


Fig. 1. Agar-cup plate tests for bactericidal activity of protein showing zone of inhibition around the cups with *Staphylococcus aureus* (A) and *Eberthella typhi* (T).

Careful examination with a hand lens showed either films of growth or small colonies within these zones. Another point of interest is that around the zone of inhibition there was, in the case of each organism, a narrow ring of very heavy growth that would indicate stimulation of cell multiplication by this protein at very low concentrations. The presence of growth films in the inhibited zones and the narrow rings of stimulated growth are shown in Figure 1.

Positive results by this agar-cup plate method are usually considered to be indicative of actual bactericidal activity. Thus, on the basis of the results obtained, true bactericidal action for both Gram-positive and Gram-negative bacteria can be claimed. Possibly the lower pH of the nutrient agar employed in these tests, as compared to that of the nutrient beef-heart infusion and glucose broth used in the previous tests, was responsible for the greater bactericidal activity shown.

Indications obtained by the agar-cup plate method of actual bactericidal activity were confirmed by results of tests using the accelerated death curve method of Cade and Halvorson (1934). In these studies the two test organisms *Staphylococcus aureus* and *Eberthella typhi* were again employed. The procedure was as follows:

Solutions to be tested were made up by dissolving the crystalline protein hydrochloride in sterile distilled water to give concentrations equivalent to 1.0, 0.5, 0.25, 0.10, 0.05, 0.01, 0.005 and 0.001 mg per ml. These solutions and control solutions of sterile distilled water were transferred to sterile, lipped 25 × 150-mm "Pyrex" test tubes. These tubes were then suspended in a constant-temperature water bath at 30°C and allowed to come to that temperature. Prior to use, the test cultures were passed through a series of three daily transfers in plain broth with incubation at 37.5°C. Transfers to the test solutions were made with a standard loop needle. Using the same standard loop, the tubes containing the protein hydrochloride and test organisms were sampled at intervals of 2, 5, 10, 20, and 30 minutes. These loop subsamples were transferred to tubes containing 15 ml of sterilized nutrient agar maintained at 45°C. Immediately thereafter the contents of these tubes were poured into sterile petri dishes and the agar was allowed to solidify. As soon as this occurred the plates were placed in an incubator at 37.5°C for 48 hours. Counts were made using a Quebec colony counting chamber. The counts obtained are listed in Table III.

TABLE III
BACTERICIDAL ACTION OF CRYSTALLINE PROTEIN IN WATER SOLUTION
AS SHOWN BY METHOD OF CADE AND HALVORSON

Con- centration of protein hydro- chlo- ride	Bacterial count per standard loop sub- sample with <i>Staphylococcus aureus</i>						Bacterial count per standard loop sub- sample with <i>Eberthella typhi</i>					
	Exposure period in minutes						Exposure period in minutes					
	0	2	5	10	20	30	0	2	5	10	20	30
mg per ml												
1.0	4,160 ¹	58	12	3	3	2	4,820	354	232	107	80	38
0.5	4,160	55	19	7	6	5	4,820	352	182	98	82	49
0.25	4,160	67	23	11	9	4	4,820	224	162	88	34	22
0.10	4,160	72	44	27	19	14	4,820	576	148	87	27	20
0.05	4,160	54	33	24	5	5	4,820	402	308	22	19	19
0.01	4,160	87	18	6	6	1	4,820	4,600	3,600	1,900	1,200	320
0.005	4,160	218	42	24	8	6	4,820	—	—	—	—	—
0.001	4,160	3,420	2,580	2,240	1,870	670	4,820	—	—	—	—	—
None	4,160	3,840	3,710	3,710	3,580	3,560	4,820	4,640	4,610	4,490	4,460	4,460

¹ Each figure is the average of two or more determinations. Zero counts were recorded occasionally but could not be duplicated.

A greater activity is shown against the Gram-positive organism *Staphylococcus aureus* than the Gram-negative organism *Eberthella typhi*. With the former, killing of cells occurred in dilutions as low as 0.005 mg per ml, whereas the lowest concentration which showed appreciable killing with *Eberthella typhi* was 0.05 mg per ml. Also, at equivalent concentrations showing death of cells with both organisms a greater percentage of the cells of *Staphylococcus aureus* was killed than with *Eberthella typhi*.

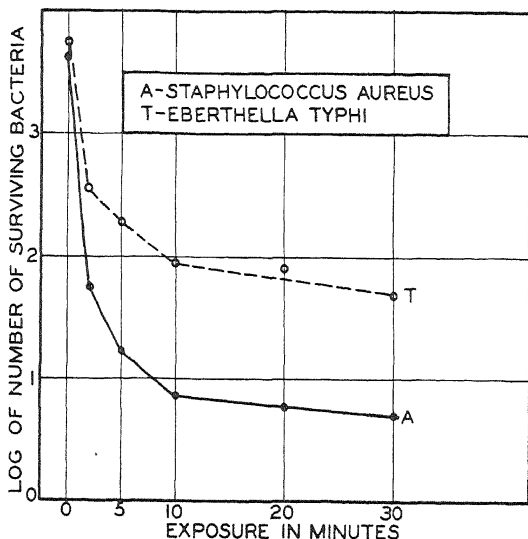


Fig 2 Logarithmic survival curves of *Staphylococcus aureus* and *Eberthella typhi* in solutions of wheat flour protein at 0.5 mg per ml.

In Figure 2 the logs of the numbers of surviving bacteria after the various exposure intervals to solutions containing 0.5 mg per ml of the crystalline protein hydrochloride are plotted. These curves give a clear picture of the comparative activity of the protein solutions against the two test organisms. It would appear from the data in Table III that the logarithmic survival curves remain the same for each organism over the range of protein concentrations that give any appreciable killing effects. This characteristic is rather unusual.

"In Vivo" Bactericidal Tests

With bactericidal polypeptides Dubos and Cattaneo (1939) were unable to find any positive correlation between "in vitro" and "in vivo" activity. It seems desirable, therefore, to conduct "in vivo"

studies. In these tests *Pneumococci Types I and III*, *Streptococcus viridans*, and *Streptococcus epidemicus*⁴ (Beta type of hemolysis) were used as test organisms. White Swiss mice between eight and ten weeks old were employed. They weighed 20 ± 0.2 g each. All inoculations and injections were made into the peritoneal cavity. With the strains of pneumococci, virulence was first built up by serial passages through mice. Saline suspensions of organisms in peritoneal washings from mice inoculated 18 hours previously were used for

TABLE IV
RESULTS OF "IN VIVO" BACTERICIDAL TESTS

Test organism and minimal lethal dose per mouse	Number of mice inoculated	Size of inoculation per mouse	Amount of protein hydrochloride given	Results ¹					
		ml	mg						
<i>Pneumococcus</i> Type I, 0.0001 ml	6	0.2	0	D44	D48	D96	D96	D96	D108
	6	0.2	0.01	D48	D72	D96	D96	D132	D132
	6	0.2	0.02	D36	D36	D44	D72	D72	D96
<i>Pneumococcus</i> Type III, 0.00001 ml	6	0.2	0	D44	D68	D72	D48	D48	D72
	6	0.2	0.01	D44	D44	D84	D44	D44	D68
	6	0.2	0.02	D44	D44	D44	D44	D44	D44
<i>Streptococcus</i> <i>viridans</i> , not virulent even in 0.5 ml quantity	3	0.5	0	All mice survived. All mice survived. D72 D84 D96					
	3	0.5	0.01						
	3	0.5	0.02						
<i>Streptococcus</i> <i>epidemicus</i> , 0.001 ml	3	0.3	0	D72	D72	D112			
	3	0.3	0.01	D72	D112	D184			
	3	0.3	0.02	D44	D72	D72			

¹ The letter "D" indicates death, and the number following the hour at which death occurred or as soon thereafter as the animal was found dead.

inoculation of test animals. With the streptococci, inoculations were made directly from 24-hour cultures washed from horse blood agar slants. The toxicity of the protein and the virulence of the four organisms were first determined.

It was found that 0.3 mg per mouse was the minimal lethal dose of the protein. Although those receiving 0.2, 0.1, and 0.05 mg suffered severe shock, they survived. Those receiving 0.02, 0.01, and 0.005 mg showed only very mild reactions and quickly recovered. On the basis of these studies 0.02 and 0.01 mg were the maximum amounts of the protein that could be designated as safe for use in the "in vivo" tests.

⁴ The identity of this organism is not certain. It resembled closely a culture of *S. epidemicus* but was a freshly isolated strain from a case of tonsillitis.

Mice were inoculated with the test organisms and immediately thereafter, within two minutes, the protein in physiological saline solution was injected. These tests, therefore, should measure any protective influence of one dose of the protein against an infective dose of the test organisms. The results of these studies are summarized in Table IV.

From the data in Table IV it is apparent that the protein has no "in vivo" bactericidal activity comparable to that found by Dubos (1940) and Dubos and Cattaneo (1939) for the peptide "gramicidin." It would even appear that in the amounts given the test animals were rendered more susceptible to infection. The average death time for mice receiving the protein was shorter with both *Pneumococcus Type I* and *Pneumococcus Type III* and *Streptococcus epidemicus* than with control mice. With *Streptococcus viridans* the only animals in the tests that died were those that received 0.02 mg of the protein.

Studies with True Yeasts

Inasmuch as the protein substance under investigation was found in wheat flour it seemed desirable to determine its effect on baker's or bread yeast. Fulmer, Nelson, and Sherwood's (1921) basal salt-sugar medium (F) ⁵ was distributed in 100-ml quantities in Erlenmeyer

TABLE V
EFFECT OF PROTEIN ON STRAINS OF *Saccaromyces cerevisiae* IN MEDIUM (F)

Origin of culture	Test No.	Number of cells in inoculum ¹	Dilution plate count per ml— mg of protein hydrochloride added per ml								
			0.1	0.05	0.025	0.01	0.005	0.0025	0.001	0.0005	0.000
		<i>per ml</i>									
Bakery	1	1,780	0	0	0	0	0	3,400	14,800	97,000	203,000
	2	1,780	0	0	0	0	100	2,200	21,700	109,000	219,000
	Average	1,780	0	0	0	0	50	2,800	18,250	103,000	211,000
Brewery	1	5,670	0	0	0	0	0	10	520	31,000	370,000
	2	5,670	0	0	0	0	0	20	730	44,000	660,000
	Average	5,670	0	0	0	0	0	15	625	37,500	490,000
Winery	1	7,830	0	0	0	0	0	0	800	180,000	290,000
	2	7,830	0	0	0	0	0	0	210	210,000	340,000
	Average	7,830	0	0	0	0	0	0	510	195,000	310,000

¹ Determined by dilution plate method using wort agar.

flasks of 200 ml capacity and sterilized in 15 pounds of steam for 20 minutes. Amounts of the protein hydrochloride were added to give a series of concentrations equivalent to 0.1, 0.05, 0.025, 0.01, 0.005, 0.0025, 0.001, and 0.0005 mg per ml. Each of these flasks and of the control flasks of medium without added protein was then inoculated

⁵ Water 1,000 ml; NH₄Cl 1.88 g; K₂HPO₄ 1.0 g; CaCl₂ 1.0 g; sucrose 100.0 g; pH 5.5.

with 1 ml of a physiological saline suspension of cells of *Saccaromyces cerevisiae* (bakers' strain). All flasks were incubated for 24 hours at 30°C. Dilution plate counts were then made using wort agar. These plates were incubated for 72 hours at 30°C prior to counting.

Similar studies were made with two other strains of *Saccaromyces cerevisiae* used industrially—one by a brewery and the other by a winery. The results of these studies are given in Table V.

The counts in Table V show that the protein is extremely toxic to the strains of *Saccaromyces cerevisiae* used. It apparently killed the inoculum in concentrations of 0.005 mg per ml and higher. It prevented any appreciable multiplication of the inoculum in concentrations as low as 0.001 mg per ml. It would appear, therefore, that this material is considerably more active in killing and inhibiting yeast growth than bacterial growth. For, although 0.005 mg per ml killed a high percentage of the cells of *Staphylococcus aureus*, it did not kill them all and it demonstrated little if any killing action against *Eberthella typhi*. In low concentration, then, this material might be described as a specific yeast poison.

Studies with Pathogenic Fungi of a Yeast-like Nature

In view of the activity of this protein in killing and inhibiting yeast growth, tests were made to determine whether it would demonstrate a like activity against the so-called pathogenic yeasts such as *Debaryomyces nadsiformis* (*torula histolytica*) and *Endomycopsis albicans* (*monilia albicans*).

Exactly the same technique was employed in these studies as with *Saccaromyces cerevisiae* except that medium (F) was modified to contain 10 g of malt extract and 2 g of bacto-peptone per liter. Each

TABLE VI
EFFECT OF PROTEIN ON GROWTH OF *Debaryomyces nadsiformis*
AND *Endomycopsis albicans*

Test organism	Test no.	Number of cells in inoculum	Dilution plate count per ml—mg of protein hydrochloride added per ml								
			0.1	0.05	0.025	0.01	0.005	0.0025	0.001	0.0005	0.000
<i>Debaryomyces nadsiformis</i>		per ml									
	1	88,000	0	0	0	0	0	0	0	4,400,000	18,400,000
	2	88,000	0	0	0	0	0	0	0	1,080,000	11,100,000
	Average	88,000	0	0	0	0	0	0	0	2,740,000	14,750,000
<i>Endomycopsis albicans</i>	1	99,000	0	0	19	82	120	700,000	16,000,000	35,200,000	41,400,000
	2	99,000	2	3	11	60	260	500,000	12,000,000	19,100,000	37,600,000
	Average	99,000	1	1.5	15	71	190	600,000	14,000,000	27,150,000	39,500,000

flask was inoculated with 1 ml of a saline suspension of cells washed from seven-day wort slant cultures. The results of these studies are given in Table VI. The data given in Table VI show that there is a marked toxicity for these two pathogenic yeasts. *Debaryomyces nadiformis* is completely killed at concentrations of 0.001 mg per ml and above. *Endomycopsis albicans*, although not completely killed, showed very low counts at concentrations of 0.005 mg per ml and higher, indicating that the protein had a definite killing activity for most of its cells.

Studies with Mycelial Fungi

To determine if the toxicity for true yeasts and pathogenic fungi of a yeast-like nature could be interpreted as a general fungicidal activity, tests were made using strains of the common mycelial fungi *Aspergillus niger* and *Rhizopus nigricans*. For these tests potato infusion-dextrose agar at pH 5.6 was employed. This agar was bottled in 100-ml quantities and sterilized. It was then cooled in a constant-temperature water bath to 45°C. As soon as the agar had come to this temperature, the protein hydrochloride was added to bring its concentration in the various bottles to 0.5, 0.2, 0.1, 0.05, 0.02, 0.01, and 0.005 mg per ml. Plates made from the agars containing varying amounts of the protein hydrochloride and control plates made with agar containing no added protein were then inoculated in the center with a few conidiospores of the test organisms. All plates were incubated at 30°C.

The results of these studies showed no fungicidal action as far as the mycelial fungi used are concerned. Thus fungicidal activity with this protein would appear to be confined to yeasts and yeast-like organisms.

Lytic Activity

In the nutrient-broth bactericidal tests with this protein the only organism completely killed was *Sarcina lutea*, a bacteria notorious for its susceptibility to certain naturally occurring lytic agents. Thus it seemed essential to determine if the ability of this protein to kill bacteria and yeast cells could be directly correlated with an ability to dissolve the cell walls. Consequently, "in vitro" studies were made with red blood cells (equine); with bacterial cells, *Sarcina lutea*; and with yeast cells, *Saccaromyces cerevisiae*. The red blood cells were separated from defibrinated blood by centrifugation and washed three times in physiological saline prior to use. *Sarcina lutea* was grown on plain agar slants for 48 hours. The cells were then washed with saline and separated by centrifugation. They were also washed three times before being used. The cells of *Saccaromyces cerevisiae* were taken

from 72-hour wort-agar slants, separated and washed for use in the same manner as employed with *Sarcina lutea*.

All three types of cells were suspended in 1.0-ml quantities (of wet mass) in tubes containing 9.0 ml of physiological saline with 0.1, 0.05, 0.025, 0.01, and 0.005 mg per ml of added protein hydrochloride. All tubes containing red blood cells, bacterial cells, and yeast cells suspended in these solutions were incubated at 37.5°C for six hours. They were then examined macroscopically and microscopically for signs of cell lysis. In no case was there any evidence of lytic activity. The results make it apparent that although this protein may have surface activity which will aid a lytic agent when present, it is not a lytic agent in itself and brings about the death of cells by some other means.

Discussion

It has been known for a long time that in cereals some substances are present which prevent fermentation by yeasts. It has been stated that as early as 1895 Jago reported that added wheat flour would inhibit beer-yeast fermentations. Hoffman (1907) attributed this inhibition of yeast activity to substances having the characteristics of albuminous breakdown products. Lindet (1910), Lange (1907), and Henneberg (1908) found that both wheat and rye contained substances that actually killed beer yeast. Baker and Hulton (1910), Lecourt (1928), Hayduck (1909), and Mohs and Kühn (1929) all attempted to separate or to define indirectly the substance in wheat flour possessing this activity. Their results showed in general a material having activity in very high dilution with toxic amine characteristics.

From the results of the studies reported in this present paper it would appear that the protein separated and crystallized as the hydrochloride by Balls, Hale, and Harris (1942) is in all probability the substance that gives wheat flour its yeast-killing activity. It is active in very high dilutions. It is strongly basic in nature, containing 20% of the basic amino acid arginine. Thus, its activity might easily be interpreted as that of a toxic amine when studied in unpurified preparations.

This protein in flour and meals has at times caused serious trouble to millers and bakers since, if present in high concentration, it acts to prevent or interfere with the rising of bread dough. The separation and purification of this material appear to constitute a major step toward the control of this problem. If, as seems likely, rye and barley contain the same or similar proteins the development of laboratory and industrial methods for removal or inactivation of such proteins should interest both distillers and brewers.

It is of special interest to note that Lecourt (1928), working with samples of wheat flour, found that the yeast-poisonous principle present had no influence on yeast zymase although it killed the cells. Should this prove to be the case with the purified protein used in these studies, this protein should serve as a valuable tool for the enzyme chemist in his studies on the enzyme complexes of yeast cells.

The activity in killing the pathogenic yeast *Endomycopsis albicans* suggests a possible therapeutic use for this protein in the treatment of tropical sprue. It might also have value in the treatment of blastomycosis where yeast-like organisms are involved. Further, its potential value as a fermentation control or aid where yeast growth is undesirable should not be overlooked.

It should be emphasized that the experiments reported herein are exploratory in nature and that results obtained under conditions differing from those specified may not be similar. A great deal remains to be done relative to the effects of pH, electrolytes, and organic materials on the activities reported herein.

Summary

Studies of the bactericidal and fungicidal activity of a protein separated from unbleached wheat flour have been made, using the protein in the form of the purified, crystallized hydrochloride. This protein has both bactericidal and bacteriostatic activity "in vitro." No "in vivo" activity could be demonstrated when tested in mice against pneumococci and streptococci. "In vitro" activity was greatest against Gram-positive organisms but there was also some activity against Gram-negative organisms.

When tested against *Saccaromyces cerevisiae* this protein was also found to possess a marked fungicidal activity, bringing about the death of the yeast cells in concentrations of 0.005 mg per ml and higher. Similar results were obtained with the pathogenic fungi *Debaryomyces nadiformis* and *Endomycopsis albicans*. However, tests using mycelial fungi failed to show any fungicidal activity. Thus, the fungicidal activity of this protein seems to be restricted to yeasts and yeast-like types of fungi.

"In vitro" tests failed to show any hemolytic, bacteriolytic, or yeast-cell lytic action. Thus, this protein cannot be classified as a lytic agent.

The industrial significance of this protein to millers, bakers, brewers, and operators of industrial fermentations has been discussed briefly.

Literature Cited

- Baker, J. L., and Hulton, H. F. E.
1910 The toxins in cereals. *J. Inst. Brew.* **16**: 395-409.
- Balls, A. K., Hale, W. S., and Harris, T. H.
1942 A crystalline protein obtained from a lipoprotein of wheat flour. *Cereal Chem.* (previous paper, this issue)
- Brewer, J. H.
1940 A clear liquid medium for the aerobic cultivation of anaerobes. *J. Bact.* **39**: 10.
- Cade, A. R., and Halvorson, H. O.
1934 Germicidal detergents: the synergistic action of soaps on the germicidal efficiency of alkalies, I and II. *Soap*: Aug., p. 17; Sept., p. 25.
- Dubos, R. J.
1940 The effect of specific agents extracted from soil microorganisms upon experimental bacterial infections. *Ann. Int. Med.* **13**: 2025-2037.
— and Hotchkiss, R. D.
1941 The production of bactericidal substances by aerobic sporulating bacilli. *J. Exp. Med.* **73**: 629-639.
— and Cattaneo, C.
1939 Studies on a bactericidal agent extracted from a soil bacillus. III. Preparation and activity of a protein-free fraction. *J. Exp. Med.* **70**: 249-256.
- Fulmer, E. I., Nelson, V. E., and Sherwood, F. F.
1921 The nutritional requirements of yeast. I. The role of vitamins in the growth of yeasts. *J. Am. Chem. Soc.* **43**: 186-191.
- Hayduck, F.
1909 Über einen Hefengiftstoff in Hefe. *Wochschr. Brau.* **26**: (73) 177.
- Henneberg, W.
1908 Über den Einfluss von Mehl und Anderen Stickstoffhaltigen, Salzen und Säuren auf die Lebensdauer und Gärkraft der Hefen in destillierten Wasser mit Rohrzucker und in Würzen. *Wochschr. Brau.* **25**: (6) 77
- Hoffman, J. F.
1907 Einige Betrachtungen über die Keimfähigkeit von Getreide und Anderen Samen. *Wochschr. Brau.* **24**: (18) 239.
- Hotchkiss, R. D., and Dubos, R. J.
1940 Bactericidal fractions from an aerobic sporulating bacillus. *J. Biol. Chem.* **136**: 803-804.
- Lindet, P.
1910 Through Untersuchungen über die Giftigkeit des Weizenmehls gegen Hefe. (See Lecourt, R.) *Ann. brasserie* **13**: 156.
- Lecourt, R.
1928 Untersuchungen über die Giftigkeit des Weizenmehls gegen Hefe. *Wochschr. Brau.* **45**: Part I, No. 11, 123-126 and Part 2, No. 14, 160-162.
- Lange, H.
1907 Über den physiologischen Zustand der Hefe. *Wochschr. Brau.* **24**: No. 32, 417-421.
- Mohs, K., and Kühl, H.
1929 Über die Wirkung von Mehl auf die Gärkraft der Hefe. *Z. Untersuch. Lebensm.* **57**: 443-448.
- Reddish, G. F.
1929 Methods of testing antiseptics. *J. Lab. Clin. Med.* **14**: 649-658.
- Ruhle, G. L. A., and Brewer, C. M.
1931 United States Food and Drug Administration methods of testing antiseptics and disinfectants. *U. S. D. A. Circ. No. 198*, p. 20.

EFFECT ON SMALL LABORATORY ANIMALS OF THE INJECTION OF THE CRYSTALLINE HYDRO- CHLORIDE OF A SULFUR PROTEIN FROM WHEAT FLOUR *

E. J. COULSON,¹ T. H. HARRIS,² and B. AXELROD²

U. S. Department of Agriculture, Washington, D. C.

(Received for publication October 9, 1941)

In previous papers from the Enzyme Research Laboratory Balls and Hale (1940) reported the occurrence of a sulphydryl compound of protein-like nature in the petroleum-ether extract of wheat flour and Balls, Hale, Harris (1942) reported the chemical examination of a crystalline protein obtained from this petroleum-ether extract. More recently Stuart and Harris (1942) showed that the crystals (as well as the partly purified material from which they were obtained) had a powerful killing action against some, but not all, species of bacteria.

In view of the toxicity toward bacteria, it is of interest to learn if animals could be protected by this substance against inoculation with disease-producing microorganisms, as Dubos and Cattaneo (1939) succeeded in protecting mice against pneumococcus by injections of gramicidin. It was found that the material derived from wheat is itself toxic to animals when injected either intraperitoneally or intravenously. When given by mouth, however, relatively large doses were found to be harmless. The behavior of the substance is thus similar to what has been frequently observed in other bodies of protein-like structure and relatively low molecular weight, such as protamines and peptones.

The symptoms observed after injection were usually labored respiration, followed (especially in mice) by loss of equilibrium. This was succeeded by an apparent coma, which may have been paralysis with the muscles relaxed. Thereafter death or recovery occurred. In rabbits and guinea pigs, however, a large but sublethal dose sometimes produced no symptoms at all, whereas mice usually showed distress with such doses although they eventually recovered. Because most gross symptoms of toxicity other than death cannot be tabulated satisfactorily, the reaction following injection has been described in the tables merely as none, slight, moderate, or severe.

The activity of the crystalline hydrochloride was further studied by the Schultz-Dale technique on the isolated uteri of virgin guinea pigs. It was found that the substance contracted the normal uterus, tracing

* Joint contribution from the ¹Allergen Investigations Division and the ²Enzyme Research Laboratory of the Bureau of Agricultural Chemistry and Engineering. Work done under Special Research Funds provided by Bankhead-Jones Act.

a curve similar to that obtained by Rocha e Silva (1940) with trypsin. Unlike trypsin, however, doses of the protein did not desensitize the muscle to subsequent doses thereof. The sensitivity of the uterus to the protein was greatest in Tyrode solutions containing 0.025 to 0.05 g calcium chloride per liter. Contraction was usually slight or entirely inhibited in calcium-free Tyrode or in solutions containing 0.075 g or more of calcium chloride per liter. The smallest dose of the protein to contract the uterus maximally was found to be of the order of 1 part in 1,250,000. Onset of contraction was slower than that produced by small doses of histamine but contraction was sustained much longer with the protein. The present paper reports the experiments on which the foregoing statements are based.

Experiments on mice: Swiss white mice¹ between eight and ten weeks old and weighing 20 g each were injected intraperitoneally with

TABLE I
INTRAPERITONEAL INJECTION OF MICE WITH SOLUTION
OF THE CRYSTALLINE HYDROCHLORIDE

Amount injected	Reaction following injection
mg	
0.50	Died in 40 minutes.
0.30	Died in 60 minutes.
0.20	Severe; recovered in 2 hours.
0.10	Severe; recovered in 18 hours.
0.050	Severe; recovered in 18 hours.
0.010	Slight; recovered in 18 hours.
0.005	Slight; recovered in 18 hours.

a solution of the crystalline hydrochloride in physiological salt solution. The volume injected varied between 0.05 ml and 0.5 ml. The results given in Table I refer individually to each animal used and show the weight of crystals received by that animal.

A similar experiment was then made using material that had been hydrolyzed by acid as is commonly done with proteins. 10.6 mg of protein hydrochloride in 0.3 ml of 20% HCl was boiled under a reflux condenser for 5 hours. The liquid was then neutralized with sodium bicarbonate and diluted to 10 ml with water. Appropriate amounts were injected with the results shown in Table II.

From these experiments, it appears that the minimum lethal dose of the original hydrochloride for mice is about 15 mg per kilo of body weight when injected intraperitoneally. Acid hydrolysis for five hours greatly reduced the toxicity, but did not altogether destroy it.

Experiments on guinea pigs and rabbits: The results of intraperitoneal injection in guinea pigs and rabbits are given in Table III for

¹ We are indebted to Mr. L. S. Stuart of this Bureau for supervising the experiments with mice.

TABLE II
INJECTION OF MICE WITH THE SUBSTANCE AFTER ACID HYDROLYSIS

Amount injected	Reaction following injection
mg	
1.00	Severe and immediate; complete recovery in 18 hours.
0.60	" " " " " " 18 "
0.60	" " " " " " 18 "
0.50	" " " " " " 18 "
0.50	" " " " " " 18 "
0.30	Severe in 20 minutes, " " " 18 "
0.30	" " 20 " " " 18 "
0.20	" " 20 " " " 18 "
0.20	" " 20 " " " 18 "

each animal used. The preparation injected was an aqueous solution neutralized to litmus with sodium hydroxide. The volume injected varied between 0.25 and 0.50 ml. The experiments are not sufficient in number to permit an estimate of the fatal dose, but they do indicate that for guinea pigs this dose is of the same order of magnitude as for mice. Rabbits appear to be somewhat more resistant.

TABLE III
INTRAPERITONEAL INJECTION OF GUINEA PIGS AND RABBITS

Weight of animal	Dose	Reaction following injection
g	mg per kilo of body weight	
GUINEA PIGS		
304	6.6	None observed.
346	5.8	None observed.
365	5.5 + 5.5 in 17'	Moderate distress, died 2 days later.
318	6.3 + 6.3 in 4'	None observed; died following day.
320	12.8	None in 1 hour; died following day.
354	11.4	None observed
RABBITS		
2136	0.7	None observed.
1528	5.2	None observed.
1749	8.9	None observed
1595	13.8	Slight, normal in 9'.
1410	12.0	None observed.

Table IV shows the results of intravenous injection in rabbits and guinea pigs. The material was dissolved in water and the solution neutralized to litmus with sodium hydroxide. The concentrations were so chosen that the volume of liquid injected into the animal was 0.2-0.4 ml. It is evident that an intravenous dose of 1.6 mg per kilo of body weight would be fatal to guinea pigs in about half the cases. Rabbits are probably more resistant than guinea pigs but the number

TABLE IV
INTRAVENOUS INJECTION OF GUINEA PIGS AND RABBITS

Weight of animal g	Dose mg per kilo of body weight	Reaction following injection
GUINEA PIGS		
330	4.7	Dead in 1 minute.
304	4.0	Dead in 1 minute.
335	3.7	Dead in 10 minutes.
316	2.2	Dead in 10 minutes.
415	2.2	Dead in 10 minutes.
367	2.2	Dead in 5 minutes.
314	1.7	Severe; recovery complete.
402	1.6	Dead in 17 minutes.
336	1.6	Severe; recovery complete.
330	1.6	Dead in 14 minutes.
412	1.1	Moderate; recovery complete.
363	1.1	Slight; recovery complete.
324	1.1	Slight; recovery complete.
350	1.1	Moderate; recovery complete.
370	1.1	Moderate; recovery complete.
RABBITS		
1562	12.5	Dead in 10 minutes.
1810	4.6	Dead in 9 minutes.
1064	4.5	None observed.
1750	2.9	None observed.
1498	2.5	Severe; died overnight.
2000	3.0	None observed.

TABLE V
ORAL ADMINISTRATION AND SUBSEQUENT INTRAVENOUS INJECTION

Initial weight of animal (g)	349	309	387
Amount given:			
(mg)	80	32	40
(mg/kilo)	229	104	103
Symptoms within 7 days of ingestion	none	none	none
Dose injected 7 days after oral administration (mg/kilo)	2.6	1.0	19.5
Reaction following injection	(intraven.) Dead in 5 minutes	(intraven.) Slight	(intraperit.) None observed
Final weight (g)	361	327	390

of animals used was too small to permit an approximation of the fatal dose.

Oral administration: Relatively large doses of the crystalline substance (dissolved in water and neutralized with NaOH to litmus) were given to guinea pigs through a stomach tube without any apparent effect within a week. The doses were somewhat greater than 50 to 100 times the fatal intravenous dose, as shown by the preceding experiments. In each case the animals gained weight and appeared

to be quite normal. They were, however, still sensitive to intravenous injection of the same material.

Experiments with excised guinea pig uterus: The Schultz-Dale apparatus used was a modification of the apparatus employed for the assay of posterior-pituitary solutions by the U. S. Pharmacopocia method. The volume of each bath was 50 ml. Tyrode solution with reduced calcium content, as frequently employed in anaphylactic studies, made possible the use of uteri from mature animals. On the

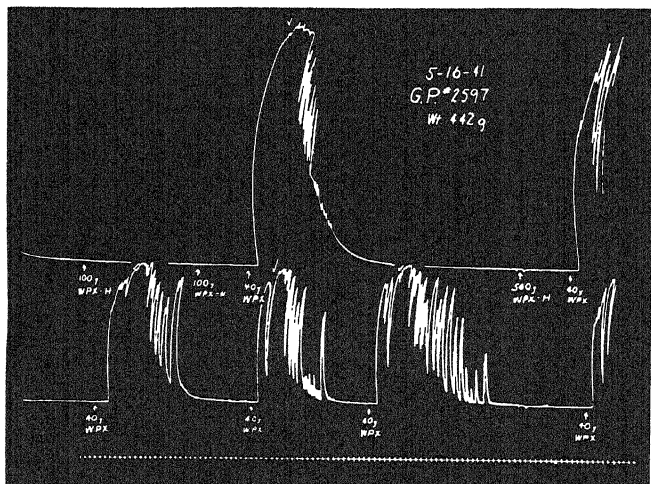


Fig. 1 Showing the effect of acid hydrolysis on the uterus-contracting property of WPX; also repeated contractions with equal quantities of WPX. The check marks (✓) above the curves indicate the point at which the bath was flushed and fresh Tyrode solution added. Time interval is in minutes.

records, reproduced as Figures 1 to 4, the symbol WPX has been used to designate the protein-like substance from wheat; the other abbreviations are self-explanatory. The amount of substances noted on the records represents the total added to the 50-ml baths.

In Figure 1 the lower curve represents the contraction of one horn of the uterus with 40 micrograms (1 part in 1,250,000) of WPX repeated three times with the same quantity of material. It is evident that the muscle does not become desensitized to WPX. The upper curve of Figure 1 shows the effect on the second horn of the uterus of WPX that has been hydrolyzed 5 hours with 20% HCl (WPX-H). Thus acid hydrolysis completely destroys the muscle-contracting property of the substance.

Large doses of the substance produce an indefinitely sustained contraction of the uterus if the bath is not flushed with fresh Tyrode

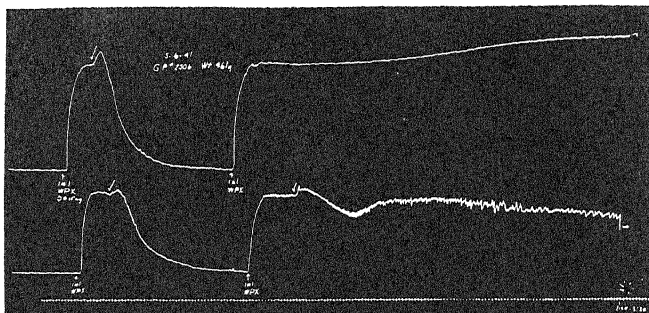


Fig. 2 Showing sustained contraction of the uterus with large doses of WPX. 1 ml solution contained 0.16 mg WPX.

solution soon after contraction has reached a maximum. This effect is shown in Figure 2.

Figure 3 shows the effect of the calcium ion concentration on the contractility of the uterus with the peptide. Concentration of calcium chloride in the Tyrode solution above 0.075 g per liter apparently inhibits contraction. Likewise contraction is inhibited in calcium-free Tyrode solution. In other experiments contraction of the uterus was

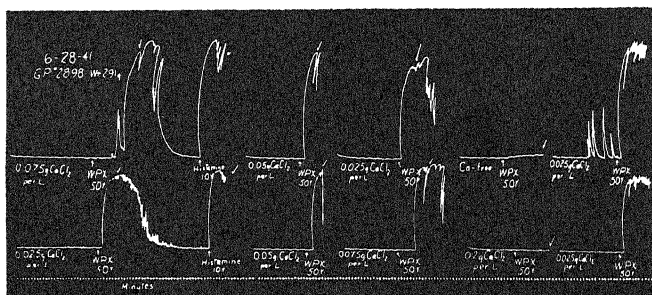


Fig. 3. Influence of calcium content of Tyrode solution on contractility of uterus by WPX.

frequently inhibited in Tyrode solutions containing 0.075 g calcium chloride per liter (Fig. 4). It has also been noted that uteri failing to contract with WPX when suspended in solutions of high calcium content will go into a sustained contraction as soon as the bath is flushed with Tyrode solution containing 0.025 g of CaCl_2 per liter.

It is well known that anaphylactic and histamine contraction will occur in Tyrode solutions over the whole range of calcium concentration from calcium-free solutions to those containing the usual quantities (0.2 g of CaCl_2 per liter). Since contraction of the uterus with the protein-like substance from wheat seems to be blocked in the

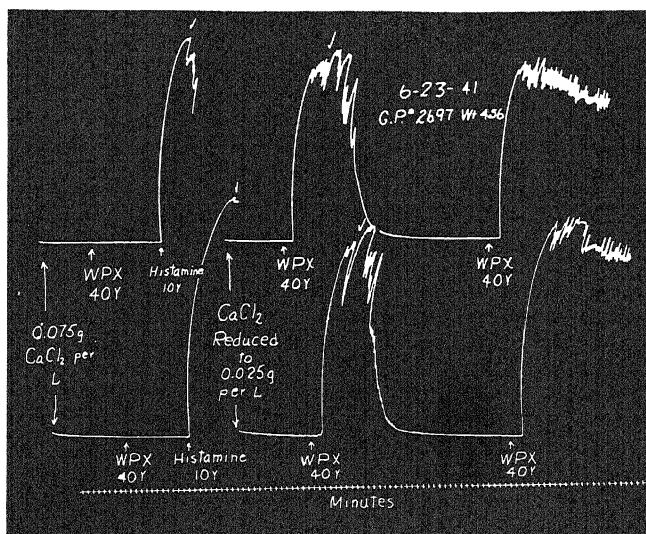


Fig. 4. Influence of calcium content of Tyrode solution on contractility of uterus by WPX.

higher ranges of calcium concentrations (and in calcium-free solutions) it would appear that either the reaction is not due to the liberation of histamine from the tissue or the reaction leading to the liberation of histamine is inhibited by the higher calcium ion concentration.

The failure of desensitization noted in the present experiments suggests the possibility that the site of action of the protein is not identical with that of trypsin, which is said by Rocha e Silva (1941) to desensitize the muscle.

Literature Cited

- Balls, A. K., and Hale, W. S.
 1940 A sulphur-bearing constituent of the petroleum ether extract of wheat flour. *Cereal Chem.* **17**: 244-245.
- Balls, A. K., Hale, W. S., and Harris, T. H.
 1942 A crystalline protein obtained from a lipoprotein of wheat flour. *Cereal Chem.*: (this issue).
- Dubos, R., and Cattaneo, C.
 1939 Studies on a bactericidal agent extracted from a soil bacillus. III. Preparation and activity of a protein-free fraction. *J. Exp. Med.* **70**: 249-256.
- Rocha e Silva, M.
 1940 Beiträge zur Pharmakologie des Trypsins. I. Wirkung des Trypsins auf die glatten Muskeln des Dünndarms und die Gebärmutter von Säugtieren. Die Freisetzung von Histamin nach Durchstromung der Meerschweinchenlunge mit Trypsin. *Arch. exp. Path. Pharmacol.* **194**: 335-350.
- Rocha e Silva, M.
 1941 Concerning the mechanism of anaphylactic and tryptic shock. *J. Immunol.* **40**: 399-404.
- Stuart, L. S., and Harris, T. H.
 1942 Bactericidal and fungicidal properties of a crystalline protein isolated from unbleached wheat flour. *Cereal Chem.*: (previous paper, this issue).

THE VARIABILITY IN THE THIAMIN CONTENT OF WESTERN CANADIAN HARD RED SPRING WHEAT OF THE 1940 CROP

H. JOHANNSON and C. E. RICH

The Ogilvie Flour Mills Co., Ltd., Montreal, Canada

(Read at the Annual Meeting, May 1941)

In literature on the subject there are many references to the "whole-wheat level" of thiamin in flour or other wheat products. This "level" is generally considered to be 1.65 International Units of thiamin per gram or 4.95 μg per gram. The word "level" implies that this figure is the average and that individual values vary only slightly from the mean.

In recent years several investigators have presented data which indicate that the thiamin content of wheats may vary within a wide range. Baker and Wright (1935) obtained values of 6.9 to 10.2 μg per g; Morgan and Hunt (1935), 3.75 and 5.67; Baker, Wright, and Drummond (1937), 3.6 to 7.8; Leong and Harris (1937), 4.5; Copping and Roscoe (1937), 3.54. Booth (1940) found that commercial samples of wheat from various parts of the world ranged from 1.62 to 7.80 μg of thiamin per gram with a mean value of 3.75. Wheats grown in England were slightly higher than this world average, varying from 2.34 to 5.94 with a mean value of 4.17. A series of 31 American wheats of various types reported by Schultz, Atkin, and Frey (1941) ranged from 4.2 to 7.3 μg , with a mean value of 5.6.

The present study was planned to determine the average thiamin content of Canadian hard red spring wheat and the variability that might be expected.

Experimental

Two hundred and sixty-five samples of hard red spring wheat of the 1940 crop were used for this study. They were samples of car lots from shipping points in Manitoba, Saskatchewan, and Alberta and were selected so as to cover this area as well as possible with a limited number of samples. The wheats were commercially graded from No. 1 Northern to No. 6 Northern, but only 14% of the samples graded lower than No. 2 Northern. The principal degrading factors in the case of the 14% were the presence of rain-bleached, starchy, frosted, or immature kernels. The weights per bushel ranged from 56 to 65 pounds, but 93% of the samples tested better than 60 pounds. Most of the samples were, therefore, sound heavy wheats.

Because the samples were taken from regular commercial car lots it was impossible or impractical to segregate them by variety. However, they were chiefly Thatcher with some Marquis, Renown, and Red Bobs. The latter variety was grown chiefly in Alberta.

Their thiamin content was determined by a thiochrome method described by Johansson and Rich (1941). Moisture, ash, and protein determinations were made according to the official A.A.C.C. methods. All the data were calculated to a 13.5% moisture basis.

The thiamin content of the samples is presented in the form of a map in Figure 1. The origin of each sample is shown as a dot and samples of similar thiamin content are designated by distinctive markings. Four groupings were chosen as indicated in the legend.

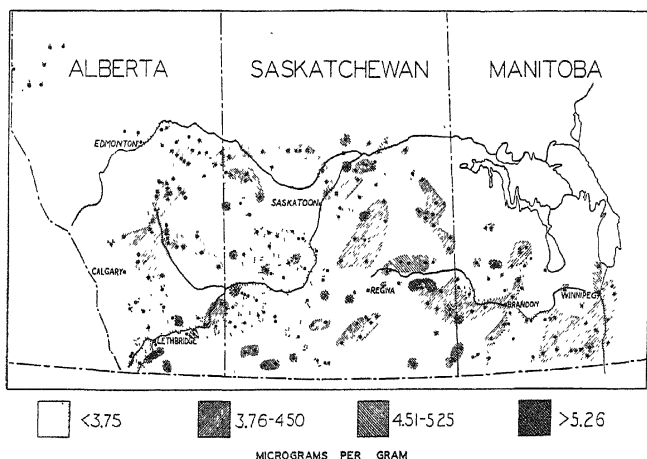


Fig 1 Areas with wheats of similar thiamin content

It is realized that to make a complete survey of an area as large as western Canada would require several thousand samples, but the volume of data is sufficient to indicate probable trends. From an inspection of the map it would appear that certain areas produce wheat of high thiamin content and that other sections produce low-thiamin wheat, but the areas are too scattered for definite conclusions. In many cases, samples of very high and very low thiamin content were found in adjacent areas.

Maps were available which showed western Canada divided into definite areas according to general soil type, and also into areas based on the ash and protein content of the wheat that each produced, but in no case did the thiamin distribution follow the outlines of any of these areas.

The summarized statistics are presented in Table I and graphically in Figures 2 and 3.

The means and range of values are almost identical with the data given by Booth (1940) on world wheats, but they are lower than those given for United States wheats by Schultz, Atkin, and Frey (1941).

TABLE I

STATISTICS ON THE THIAMIN DATA ACCORDING TO PROVINCE
Values expressed as micrograms per gram on a 13.5% moisture basis

Province	No. of samples	Mean value	Standard deviation	Range
		$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$
Manitoba	67	3.90	0.606	3.0-5.3
Saskatchewan	120	3.99	0.708	2.5-6.2
Alberta	78	3.90	0.909	2.2-8.0
All provinces	265	3.93	0.750	2.2-8.0

HISTOGRAM SHOWING THE DISTRIBUTION OF 265 WHEATS
ACCORDING TO THEIR VITAMIN B₁ CONTENTS

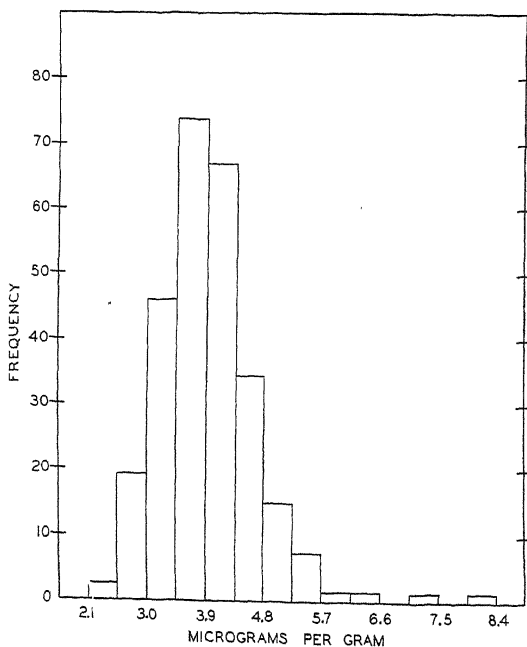


Fig. 2. The distribution of 265 wheats according to their vitamin B₁ contents.

There is no significant difference in the means of the three provinces, but the Alberta samples covered a considerably greater range in thiamin values.

The factors responsible for this large variability in thiamin content are not yet known. Data given by Booth (1940), Nordgren and

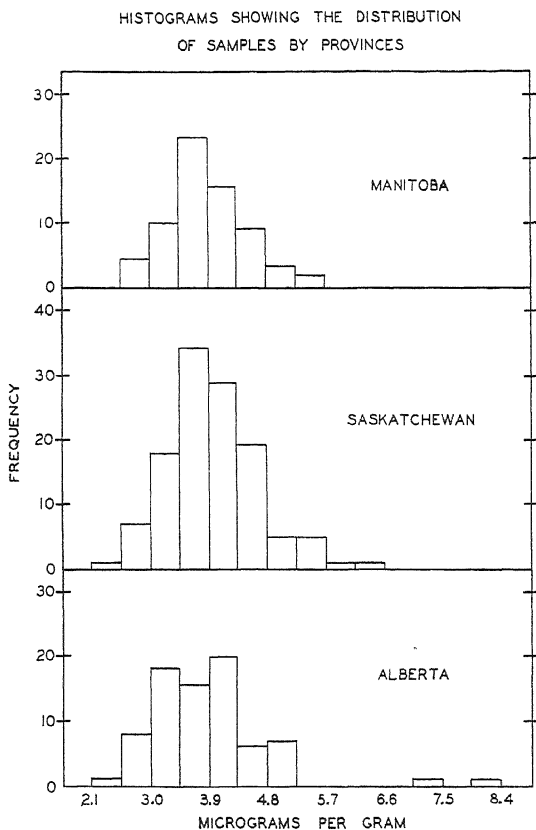


Fig. 3. The distribution of samples by provinces

Andrews (1941), and Newman (1941) indicate that variety and environment are influencing factors. Burkholder and McVeigh (1940) while studying the growth of two corn hybrids in nutrient media found that the thiamin content of the kernels varied directly with the nitrogen supply and inversely with the phosphorus in the nutrient solution. This

indicates that composition of the soil may play an important part in determining the vitamin B₁ content of the kernels. It is known that western Canadian soils vary greatly in both nitrogen and phosphorus content. This fact may account in part for the present data.

It is generally known that in a series of flours of varying grades, or within a group of mill-stream flours, there is a high correlation between thiamin content and both ash and protein content. The correlation coefficient of thiamin and the ash content in this series was +.012, and between thiamin and protein content +.161. The 5% point in both cases was +.195. Both values indicate no relationship for this series.

No relation could be found between thiamin content and weight per bushel or grade. Commercial wheats are degraded for numerous reasons—for example, because of the presence of immature, frozen, and shrunk, starchy, or otherwise damaged kernels. Unfortunately, this series was not suited to a study of these factors because most of the samples were sound and of good weight. Two samples, however, containing different degrading factors were hand picked and the normal and damaged kernels assayed separately.

TABLE II
THE THIAMIN CONTENTS OF THE NORMAL AND DAMAGED FRACTIONS
OF TWO WHEAT SAMPLES

Sample No	Commercial grade	Weight per bushel	Fraction	Thiamin content
		lbs		µg per g
86	No. 6 Northern	56	Normal kernels	3.4
			Thin and shrunk kernels	4.2
115	No. 3 Northern	64	Normal kernels	3.5
			Starchy kernels	2.9

The results obtained with sample No. 86 were anticipated because thin and shrunk kernels have a higher ratio of bran to endosperm than do normal plump kernels. The starchy kernels from sample No. 115 were somewhat lower in thiamin content than the normal kernels. Because the two fractions were grown under identical circumstances this may be an indication that conditions which produce high protein content may also produce high thiamin content.

Summary

Two hundred and sixty-five commercial hard red spring wheats from most of the crop districts in western Canada were assayed by the thiochrome method. The values ranged from 2.2 to 8.0 µg of thiamin per gram with an average of 3.93 µg per gram. The means for each prov-

ince were not significantly different, but the western part of the area studied showed a greater range in values.

The areas producing wheats of similar thiamin content appeared to have a random arrangement. No relation to soil type was observed.

The large variability observed is undoubtedly due to the influence of factors such as soil composition, climatic conditions, wheat variety, etc., and since the geographical location of the samples in this series were the only information available, these points could not be investigated. For this purpose several series of controlled experiments would be necessary to evaluate the influence of each factor on the thiamin content of wheat.

Acknowledgment

The authors are indebted to Dr. J. A. Anderson of the Dominion Grain Research Laboratory for his kind assistance in supplying many of the samples used in this study.

Literature Cited

- Baker, A. Z., and Wright, M. D.
1935 The vitamin B₁ content of foods. *Biochem. J.* 29: 1802-1807.
—, —, and Drummond, J. C.
1937 The nutritive value of bread. *J. Soc. Chem. Ind.* 56: 191-194
- Booth, R. G.
1940 The thiochrome method for the estimation of aneurin, with a survey of the aneurin content of wheats. *J. Soc. Chem. Ind.* 59: 181-184.
- Burkholder, P. R., and McVeigh, Ilda
1940 Studies on thiamin in green plants with the *Phycomyces* assay method. *Am. J. Bot.* 27: 853-861.
- Copping, A. M., and Roscoe, M. H.
1937 The water-soluble B vitamins in yeast, flour and bread. *Biochem. J.* 31: 1879-1902.
- Johannson, H., and Rich, C. E.
1941 A modified thiochrome method for the estimation of vitamin B₁ in wheat and its products. *Cereal Chem.* 18: 473-482.
- Leong, P. C., and Harris, L. J.
1937 Vitamin B₁ and the "brown versus white bread problem." II. *Biochem. J.* 31: 812-816
- Morgan, A. F., and Hunt, M. J.
1935 The vitamin B (B₁) and G (B₂) content of wheat products. *Cereal Chem.* 12: 411-418.
- Newman, L. H.
1941 Private communication.
- Nordgren, R., and Andrews, J. S.
1941 The thiamin content of cereal grains. *Cereal Chem.* 18: 802-811.
- Schultz, A. S., Atkin, L., and Frey, C. N.
1941 A preliminary survey of the vitamin B₁ content of American cereals. *Cereal Chem.* 18: 106-113.

BOOK REVIEW

The Chemical Formulary, Volume V. Edited by H. Bennett. Published by the Chemical Publishing Co., Brooklyn, N. Y. 674 pages. \$6.00.

An examination of this book gives no reason to doubt the statement in the preface that "sufficient new formulae have been gathered to compile a 5th volume . . . which will broaden and bring up-to-date the contents of Volumes I, II, III, and IV."

Volume V covers the same kinds of materials as did the previous volumes, presenting a large number and variety of formulae for the preparation and compounding of adhesives, beverages, cosmetics and drugs, farm and garden specialties, foods, materials of construction, etc. There is, however, a noticeable change in emphasis on certain types of materials. Thus, in Volume V, 146 pages are devoted to foods, as compared to 15 pages in the previous volume. In addition to numerous formulae for the preparation, processing, and preservation of all kinds of foods and food products, much up-to-date information on vitamins is presented. The second-largest section, comprising paints, enamels, varnishes, and lacquers, covers 148 pages.

Volume V carries an enlarged and convenient directory of sources of chemicals and supplies.

All who have become accustomed to consulting previous volumes of *The Chemical Formulary* will doubtless wish to have a copy of Volume V available for reference.

R. T. PRESCOTT

CEREAL CHEMISTRY

VOL. XIX

MAY, 1942

No. 3

A VISCOMETRIC DETERMINATION OF THE OPTIMUM pH FOR THE PROTEOLYTIC ACTIVITY OF MALT WITH GELATIN AS A SUBSTRATE

JOHN R. KOCH and SISTER MARY LAURETTA, S.S.N.D.

Department of Chemistry, Marquette University, Milwaukee, Wisconsin

(Read at the Annual Meeting, May 1941)

Various methods are now available for the quantitative determination of the action of proteolytic enzymes. This is followed either by observing the amount of substrate digested at the end of some given time or by determining, at intervals, the changes in the viscosity, conductivity, or turbidity of the substrate occurring during the course of the digestion effected by the enzyme (Waksman, 1926). The hydrolytic breakdown of gelatin, for example, caused by the action of the proteolytic enzymes of malt shows itself through a noticeable decrease in the viscosity of the gelatin-enzyme mixture (Ehrnst, 1938; Koch, Nelson, and Ehrnst, 1939; Northrop, 1922). The report here presented is based upon a study carried out by following such a decrease in viscosity when the pH value of the substrate was made the variable factor.

The proteolytic enzymes in malt function differently under different pH conditions (Britton, 1929; Hagues, 1924; Hopkins and Kelly, 1931). Viscosity tests show that proteolysis can be either hastened or retarded as the pH is varied (Hind, 1938; Northrop, 1923), and the pH may be varied by changing the acidity or alkalinity of the liquid in which the enzyme is present or by changing that of the solution of the product upon which the enzyme is to act (Tauber, 1937; Van Laer, 1923). Waksman (1926) and Northrop (1922) state that most enzymes are greatly influenced by the reaction of the medium in which they act, and that there is an optimal H-ion concentration for the activity of each enzyme, which accounts for upper and lower limits of reaction, above or below which the enzyme is inactive or may be rapidly destroyed.

It is well known that pepsin becomes inactive in alkaline solutions because its activity is restricted to an acid medium only. Trypsin digests proteins in either neutral or alkaline solutions, but not in an acidified medium (Northrop, 1922). Malt contains several enzymes,

and consequently its activity is spread over a wide pH range. However, each enzyme of malt has its own zone, below or beyond which it is inactivated or completely destroyed (Hagues, 1924; Mill and Linderstrøm-Lang, 1927).

Attempts to determine these zones of maximum activity have been made and a number of methods have been applied (Britton, 1929; Hopkins and Kelly, 1931; Hopkins and Krause, 1937; Lüers and Malsch, 1929; Lundin, 1923; Mill and Linderstrøm-Lang, 1927). However, none of the literature studied by the authors offers any viscometric approach to finding the optimum pH for the proteolytic enzymes of malt. Yet there is substantial evidence that more attention should be given in that direction (Ehrnst, 1938; Kolbach and Simon, 1936).

In the brewing industry the clarification and maturing of malt beverages and the correcting of protein haze is almost wholly dependent on proteolytic enzymes. Moreover, the amylase activity, so vital in malting and brewing, also depends on the preceding proteolysis (Wallerstein, 1939).

The purpose of this research was to study the viscometric determination of the optimum pH for the proteolytic activity of malt with gelatin used as a substrate. This peak activity was obtained by permitting the digestion of the substrate to occur when the pH condition of the gelatin was altered, (a) by using HCl and NaOH as modifying agents (Figs. 3, 4, and 5), and (b) by adding buffers as stabilizing factors (Fig. 6). The malts analyzed were of the commercial variety supplied to breweries and the results presented are from a study of four malts.

The viscosity measurements followed the method developed by Koch, Nelson, and Ehrnst (1939). The instrument used was a new-type, all-glass digestion-flask viscometer.

Figure 1 shows the viscometer in detail. Not only does it meet requirements for low cost, simplicity of design and operation, and adaptability to routine work, but it is also reliably accurate, and conveniently permits the measurement of viscosity at the end of any desired period in order to observe the progress of enzymatic action (Koch, Orthmann, and Degenfelder, 1939; Koch, Nelson, and Ehrnst, 1939). This viscometer as originally designed was constructed in two forms. The first had its upper and lower reservoirs held together by means of two rubber stoppers; the second was an all-glass type, which later was replaced by a more sturdy model.

In detail, the present model is assembled with glass seals throughout and serves both as a viscometer and as an almost hermetically sealed digestion flask. Its capillary, about 35 mm long, is permanently sealed

in at the junction of two 200-ml Erlenmeyers. Because the diameter of the capillary is an important factor, the bore of the one selected was such that a working volume of 50 ml of distilled water had an efflux time of approximately 30 seconds. Longer efflux time was considered unsatisfactory because the drainage time between 15-minute runs was thus somewhat shortened, and a viscous substance such as gelatin re-



Fig. 1 Viscometer.

quires more time than less viscous liquids to drain completely from the walls of a glass container. Furthermore, a fixed funnel-shaped entrance to the capillary eliminated corrections for the retention of any liquid after a run was made, in contrast to the earlier viscometer used by Nelson. The externally sealed-in side arm of the viscometer not only allowed the confined liquid to flow from one reservoir into the other at the completion of a run, but it also made the handling and inverting of the viscometer an easy routine procedure.

The viscometers were calibrated by means of a 40% sucrose and a 25% glycerol solution. By using these solutions at 25°, 30°, and 40°C the calibration was made over a wide range and carefully checked. For the details of calibration either of two previous papers may be consulted (Koch, Orthmann, and Degenfelder, 1939; Koch, Nelson, and Ehrnst, 1939). For general calibration procedure the works of Sheely (1923, 1932), Bingham and Jackson (1916), Barr (1931), and Hershel (1917) should be consulted.

Preparation of Solutions

The method of preparing a 10% stock solution of gelatin was essentially the same as that described by Koch, Nelson, and Ehrnst (1939). From this supply the 6 $\frac{2}{3}$ % concentration was obtained by dilution.

The malt infusion was prepared at 40°C by adding 20 g of malt (finely ground in a Miag-Seck mill) to 100 ml of distilled water. This was kept in the water bath (at 40°C) for 30 minutes (being stirred frequently during the time), and then filtered. One 35-ml sample of the filtrate (attempered in the water bath for about 8 minutes) was reserved for the digestion test.

The pH of the substrate was changed by selective additions of one of three agents: a buffer, HCl, or NaOH, added to the gelatin (10%) before it was made up to mark. Clark and Lubs' set of buffers was employed. These mixtures were made according to the procedure indicated by Clark (1923) and the acid and alkali were prepared in the usual laboratory way. Preliminary trials to determine the amount of buffer required to bring any particular digestion mixture to a desired pH were made prior to making any test runs. The pH of the substrate for malts 1, 2, and 3 was adjusted by adding 0.2M HCl solution or 0.2M NaOH. The buffer mixtures were added to the gelatin prepared for malt 4.

For each change of pH, a predetermined amount of buffer or acid or alkali was added to the gelatin that served as the 10% stock solution for the test under observation. The pH value recognized as the working pH of a run was the one obtained within the first five minutes after the malt extract was added to the gelatin. The pH, covering a series of tests for the malts selected, ranged from approximately 2.5 to 8.0.

Determinations and Results

Malt blank: About 35 ml of the extracted malt was placed into boiling water for 4 minutes, and then cooled rapidly. The precipitated albuminoids were filtered out and 25 ml of the filtrate was attempered to 40°. Fifty ml of gelatin (previously prepared and attempered) was now added to 25 ml of the boiled extract. Of this 6 $\frac{2}{3}$ %

mixture, 50 ml was transferred to a viscometer and run as a blank. Because the enzymes were inactivated by boiling (Koch, Nelson, and Ehrnst, 1939), the outflow time of the blank showed no appreciable variation (table showing blank for Fig. 2).

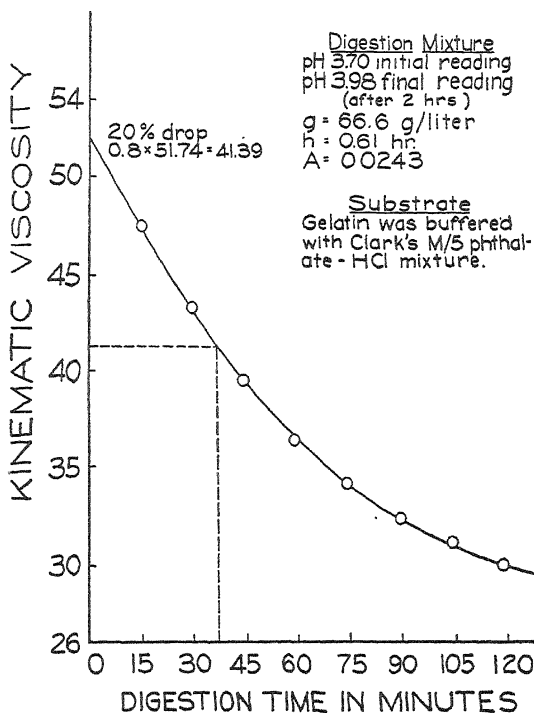


Fig. 2. Malt 4, pH 3.70.

Viscometer A—Blank		
Time intervals (min)	Viscosity reading (sec)	K (millipoise)
15	61.4	54.03
30	59.7	51.63
45	59.2	50.92
60	59.4	51.20
75	59.2	50.92
Average	59.78	51.74

Viscometer B		
Time intervals (min)	Viscosity reading (sec)	K (millipoise)
15	49.7	47.67
30	47.5	43.13
45	45.8	39.53
60	44.4	36.51
75	43.4	34.32
90	42.6	32.54
105	42.0	31.20
120	41.9	30.07

The purpose of running the blank was to establish an initial or zero point. Attempts at finding the value by extrapolating the curves resulting from the two-hour test runs proved satisfactory enough to be accepted. In fact, later in a personal conference with Mr. Lawrence Ehrnst, chief chemist of the Froedtert Grain and Malting Company, Milwaukee, Wisconsin, it was learned that he found extrapolation reliable enough for routine use in viscosity tests conducted in his

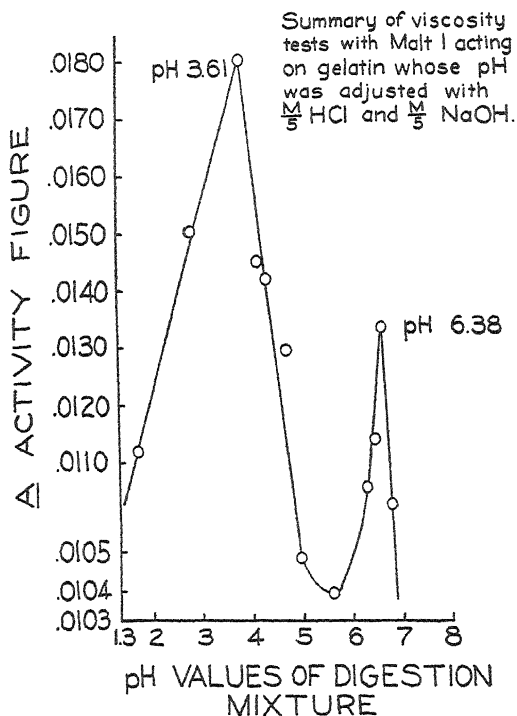


Fig. 3. Malt I

laboratories. A survey of viscosity work done by Northrop (1922, 1923) and more recently by Laufer (1937, 1938), both with gelatin as a substrate, confirms the extrapolation method for establishing a zero point. However, for the sake of strictly reproducible data as well as for the maintenance of uniformity, a blank was run in this study for every change of pH.

Viscosity determination: It was found by many comparative trials that the most efficient method of making viscosity determinations in

these experiments was to run the blank in one viscometer and the digestion mixture in another, so that both would be subjected to identical conditions of temperature and that both would have the substrate drawn from the same stock solution (freshly prepared for each change of pH), which in turn had had the same facilities for attemperation. Values were easily duplicated as a result.

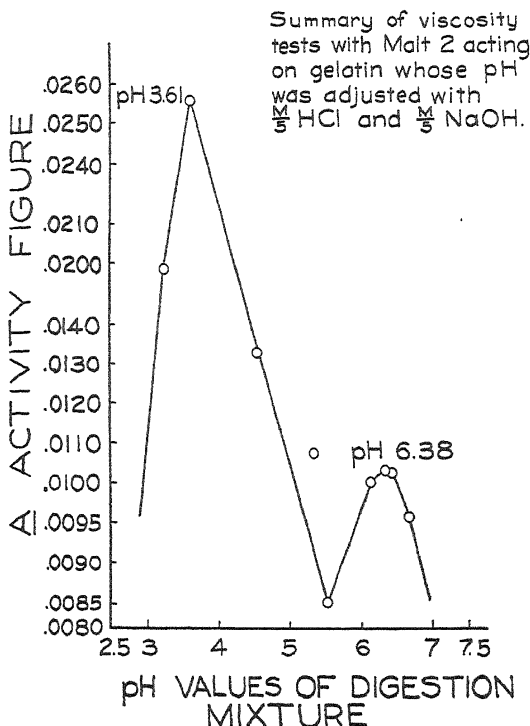


Fig. 4. Malt 2.

The time of adding 50 ml of the attemperated 10% gelatin-buffer mixture or gelatin-acid mixture to 25 ml of the unboiled extract (also at 40°) was considered the initial or zero time of the run, since digestion was begun from the moment the solutions came in contact with each other. Fifty ml of this digestion mixture was transferred to the one viscometer and 50 ml of the blank mixture was conveyed to the other. The viscometers were immersed in the water bath as soon as they were filled. At the end of 15 minutes, each viscometer was inverted in turn

and the time of flow for each was determined by timing the transit of the digestion mixture and the blank from the upper to the lower reservoir of the respective viscometer. At the completion of a run the viscometers were again put into their position of rest. Viscosity determinations were made every 15 minutes for two hours in nearly all cases for the viscometer containing the digestion mixture and for a period

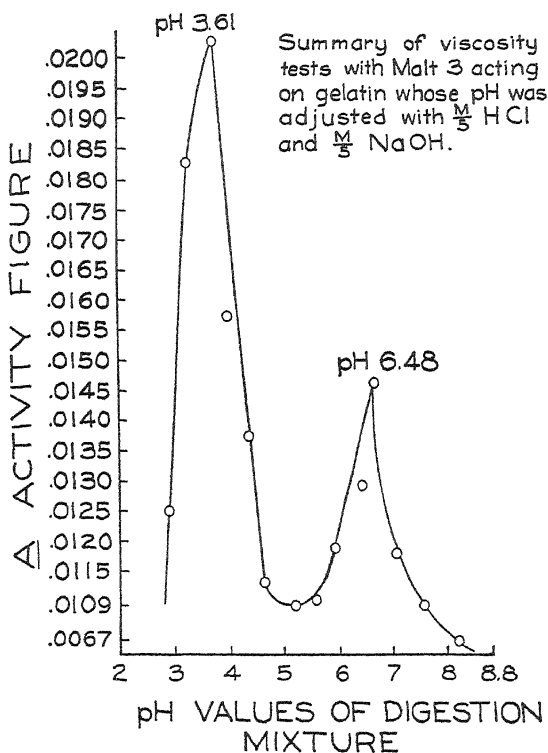


Fig 5. Malt 3.

long enough to make five trials for the blank. An average of these five trials established the initial point when the viscosity values were graphed.

Figure 2 and the corresponding numerical representation indicate a series of runs obtained at one pH. The efflux time, in seconds, of each run was converted into kinematic viscosity by consulting the calibration table (Table I made for the two viscometers).

In graphing, the kinematic viscosity values were plotted as the ordinates against the periods of digestion in minutes as the abscissas. This gave a smooth curve (Fig. 2) from which the time in minutes necessary for the proteolytic enzymes of malt to effect a 20% drop in kinematic viscosity was estimated. The point of intersection made by the horizontal dotted line (Fig. 2) with the curve for the digestion

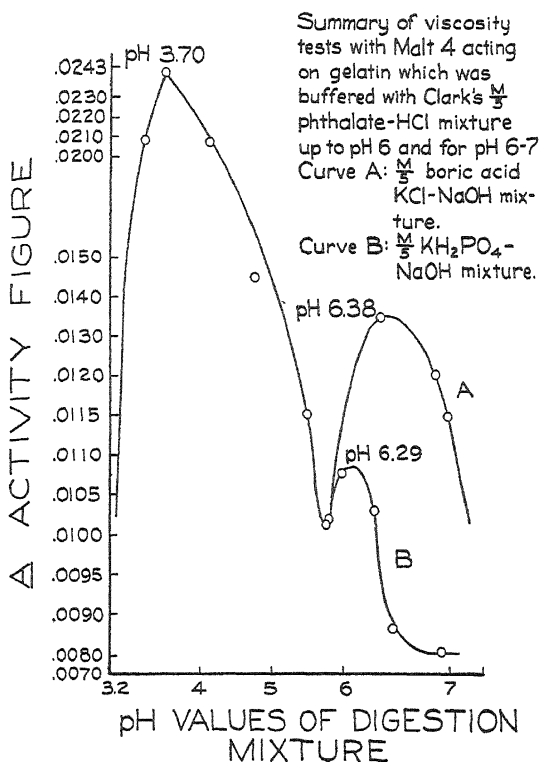


Fig. 6. Malt 4.

mixture denotes the time required to effect the 20% drop for the set pH. It is calculated by taking 80% of the viscosity of the blank. The summary graph for a series of curves (such as represented by Fig. 2) for one malt shows the rise and fall of activity as pH values of the digestion mixture are changed (Figs. 3 to 6). This graphic history of a malt operating at different pH values is obtained by plotting the pH values as abscissas and the ordinates as activity figures (*A*).

TABLE I
CONVERSION OF FLOW TIME TO KINEMATIC VISCOSITY

Viscometer A $K = At - B/t$; $A = 0.1140$; $B = 98.085$		Viscometer B $K = At - B/t$; $A = 0.1499$; $B = 133.37$	
Time in seconds	Kinematic viscosity	Time in seconds	Kinematic viscosity
40	21.08	40	26.61
41	22.82	41	28.93
42	24.53	42	31.20
43	26.21	43	33.44
44	27.87	44	35.64
45	29.51	45	37.82
46	31.12	46	39.96
47	32.72	47	42.08
60	52.06	60	67.72
61	53.47	61	69.57
62	54.86	62	71.42
63	56.26	63	72.26
64	57.65	64	75.10
65	59.01	65	76.92
66	60.38	66	78.73
67	61.75	67	80.53
68	63.10	68	82.32

Activity figures for each sample of malt were calculated from the formula, $A = 1/hg$ (Northrop, 1922), where A is the activity figure; h , the time in hours required to effect a 20% kinematic viscosity drop; and g , the concentration in grams per liter of ground malt.

Summary and Conclusions

The proteolytic activity of malt at different pH values can be measured viscometrically, thus locating an optimum pH.

A 20% drop in kinematic viscosity furnishes a desirable means of estimating proteolysis. It makes possible a determination at a given pH in two hours or less, thereby saving time and eliminating the disturbing factors that enter into prolonged digestion reactions.

A summary graph for each malt shows a wide range of pH values, indicating that the all-glass Koch viscometer is sensitive to even small changes in pH.

The amount of activity at an optimum point varies somewhat with the buffer used to produce the pH.

The use of 0.2*M* potassium acid phthalate buffer or of 0.2*M* HCl with gelatin produces the same effect. In either case there is a rise in activity with a decrease of pH—maximum occurring at pH 3.6 to 3.7.

Although only four malts are illustrated in this paper, actually ten malts were completely tested in the research. For all ten malts the maximum points found were between 3.6 and 3.7 for the one enzyme and between 6.3 and 6.5 for the other.

Acknowledgment

Appreciation is hereby extended to Mr. L. Ehrnst, Froedtert Grain and Malting Company, for all the practical assistance given this research and for the various samples of malt.

Literature Cited

- Barr, Guy
1931 A monograph of viscometry. Oxford University Press, London.
- Bingham, E. C., and Jackson, R. F.
1916 Standard substances for the calibration of viscometers. Scientific Papers of the Bureau of Standards No. 298, Aug. 8.
- Britton, H. T.
1929 Hydrogen ions. Van Nostrand Pub. Co., New York.
- Clark, W. M.
1923 The determination of hydrogen ions (2nd ed.). Williams and Wilkins Co., Baltimore, Md.
- Ehrnst, L. W.
1938 Determination of proteolytic strength in malt. *Modern Brewer* 20: 57.
- Hagues, G.
1924 The effect of hydrogen ions in brewing processes. *J. Inst. Brewing* 30: 298-304.
- Hershel, Winslow H.
1917 Determination of absolute viscosity by short-tube viscometers. Technologic Papers of the Bureau of Standards No. 100, Nov. 9.
- Hind, Lloyd H.
1938 Brewing science and practice, I. John Wiley and Sons, Inc., New York.
- Hopkins, R. H., and Kelly, H. E.
1931 The proteolytic enzymes of green malt. *Biochem. J.* 25: 256-259.
- Hopkins, R. H., and Krause, C. B.
1937 Biochemistry applied to malting and brewing. Van Nostrand Pub. Co., New York.
- Koch, J. R., Orthmann, A. C., and Sister M. Anselma Degenfelder, S. S. N. D.
1939 A digestion flask viscometer for measuring enzyme activity of bates using gelatin as a substrate. *J. Am. Leather Chem. Assoc.* 34: 489-514.
- Koch, J. R., Nelson, O., and Ehrnst, L.
1939 Viscometer for routine determination of proteolytic activity of malts. *Ind. Eng. Chem. (Anal. Ed.)* 11: 35-41.
- Kolbach, P., and Simon, H.
1936a Über die proteolytische Kraft von Malzauazugen und Wuerzen. *Wochenschr. Brau.* 53: 297-299.
1936b Proteolytic power of malt infusions and worts. *The Brewers Technical Rev.* 11: 351-353.
- Laufer, Stephen
1937 Studies on the proteolytic activity of barley malt. *J. Assoc. Offic. Agr. Chemists* 20: 307-330.
1938 Reports on proteolytic activity of malt. *J. Assoc. Offic. Agr. Chemists* 21: 160-164.
- Lüers, H., and Malsch, Ludwig
1929 Zur Kenntnis der proteolytischen Enzyme des Malzes. *Wochenschr. Brau.* 46: 265-269, 275-280.
- Lundin, H.
1923 Ein Beitrag zur Kenntnis der proteolytischen Enzyme des Malzes. *Biochem. Z.* 131: 193-218.
- Mill, C. K., and Linderström-Lang, K.
1927-29 Note on the proteolytic enzymes in green malt. *Compt. rend. trav. lab. Carlsberg* 17: 1-14.
- Northrop, J. H.
1922 The mechanism of the influence of acids and alkalies on the digestion of proteins by pepsin and trypsin. *J. Gen. Physiol.* 5: 263-274.
1923 A method for the quantitative determination of trypsin and pepsin. *J. Gen. Physiol.* 5: 353-358.
- Sheely, M. L.
1923 The standardization of commercial viscometers. *Ind. Eng. Chem.* 15: 1109-1114.
1932 Glycerol viscosity tables. *Ind. Eng. Chem.* 24: 1060-1064.

- Tauber, Henry
1937 Enzyme chemistry. John Wiley and Sons, New York.
- Van Laer, M. M.
1923 The possibilities of increasing the brewer's extract. J. Inst. Brewing 29: 202-218.
- Waksman, S. A.
1926 Enzymes. Williams and Wilkins Company, Baltimore, Md.
- Wallerstein, Leo
1939 Enzyme preparations derived from microorganisms. Wallerstein Lab. Comm. Sci. Pract. Brewing 7: 5-19.

THE DISTRIBUTION OF PHYTIC ACID IN WHEAT AND A PRELIMINARY STUDY OF SOME OF THE CALCIUM SALTS OF THIS ACID

J. G. HAY

Joseph Rank, Ltd., Reigate, Surrey, England

(Received for publication October 6, 1941)

The phytic acid phosphorus in the pericarp of the wheat grain is to a large extent fixed in the form of calcium magnesium phytate, with the result that in bran between 90% and 100% of the total phosphorus is phytic acid combined as a calcium magnesium salt of the hexaphosphoric ester of inositol. This water-insoluble salt no doubt secures the phosphorus from loss through diffusion when the grain is exposed to rain, but liberates it in an available inorganic form by action of phytase when the germinating plant requires it.

In present commercial wheat mill products the phytic acid phosphorus is directly proportional to the fiber content. With white flour, 85% wheat meal, 100% whole meal, fine wheat feed, and bran, the graph obtained when the fiber content is plotted against the phytic acid phosphorus is a straight line, as shown in Table I.

TABLE I
FIBER AND PHYTIC ACID PHOSPHORUS

	Fiber	Phytic acid phosphorus
	%	%
75% extraction white flour	0.28	0.025
85% extraction wheat meal	0.95	0.122
100% extraction whole meal	1.96	0.203
Fine wheat feed	6.10	0.660
Bran	9.75	1.105

The product referred to as fine wheat feed is the tails (from all the machines) which pass through a certain mesh, but without any additions such as ground shriveled wheat, which may be added to commercial "weatings" or sharps.

The figures given are typical of those obtained in an ordinary commercial milling of mixed wheats, with red wheats in great preponderance, as is usually the case. There is some evidence to indicate that certain white wheats may have a lower phytic acid content than red ones, as indicated in Table II.

TABLE II
PHYTIC ACID CONTENT OF RED AND WHITE WHEATS

	Fiber in wheat	Phytic acid phosphorus in wheat	Fiber in bran	Phytic acid phosphorus in bran
	%	%	%	%
White Victorian	1.95	0.17	10.58	0.78
White Pacific	2.1	0.175	10.18	0.72
Red No. 1 Manitoba	2.05	0.22	9.65	1.07

This point, if proved, is of interest, as it may account for the hitherto unexplained fact that farmers are prepared to pay a premium for white bran over red, although on analysis red bran averages a higher protein content than white, the other constituents being about the same: so possibly stock producers have found by experience that white bran gives better results in feeding stock than red, which may be due to its lower phytic acid and consequently lower calcium-immobilizing action on other foods.

One sample of white English wheat examined did not differ materially from red wheats in phytic acid phosphorus content, but before the War, when it was permissible to make "divides" in milling, by far the greater part of white bran was derived from white wheat other than English—such as Australian and White Pacific—so its influence on white bran would be small.

There are certain exceptions in the individual tails from the various centrifugals (which collectively form the fine wheat feed) in their phytic acid phosphorus content in relation to fiber content; but it must be borne in mind that the crude fiber content as determined is not a true content of cellulose, being the amount of material insoluble under certain conditions in specified strengths of acid and alkali, so that when the softer and probably more soluble tissues of the germ and the parenchymatous cells of the endosperm are compared in fiber content with those tougher structures in the pericarp, the comparison is not quite the same. However, in a product such as pure germ, as free as possible from adhering bran particles, the phytic acid phosphorus is much higher in relation to fiber content than in the case of bran, as shown in Table III.

The tails from machines containing germ stock, such as J. scalper tails, do not show a great discrepancy from the theoretical point on the

TABLE III
PHYTIC ACID PHOSPHORUS IN GERM AND BRAN

	Fiber	Phytic acid phosphorus	Ash	Total phosphorus
	%	%	%	%
1. Germ	2.15	0.52	4.41	1.12
2. Germ	2.05	0.49	4.53	0.94
Bran, same milling as germ No. 2	10.3	1.17	5.54	1.21

graph for their fiber content, for in this case the fiber content is relatively high, due to bran particles, a typical sample giving 5.35% fiber and 0.60% phytic acid phosphorus.

Another exception is found in such stocks as M. centrifugal tails. This product is a light-colored, fluffy material, containing very few bran particles; it is only a small percentage of the total fine wheat feed. Under the microscope it is found to contain a considerable number of parenchymatous cells from the interior of the endosperm. Figures for this product are shown in Table IV.

TABLE IV
DATA FOR M. TAILS

	Fiber	Phytic acid phosphorus	Ash	Total phosphorus
	%	%	%	%
M. tails	2.15	0.46	2.70	0.54

To investigate the point as to whether there was a high percentage of phytic acid in the endosperm cellular tissue, 7 pounds of purest endosperm obtainable on the mill, in the form of semolina free from bran particles, was rolled between smooth rolls on an experimental mill, the tails from a No. 11 silk were rerolled, and the tails from a similar silk, amounting to 0.23% of the semolina, were examined under the microscope and found to contain a large proportion of parenchymatous cells, as shown in Table V.

TABLE V
DATA ON ENDOSPERM

	Fiber	Phytic acid phosphorus	Total phosphorus	Calcium	Ash	Percentage of phosphorus as phytic acid phosphorus
	%	%	%	%	%	%
Tails from pure endosperm	1.15	0.435	0.437	0.076	2.04	99.5
Flour from pure endosperm	0.12	0.025	0.079	0.019	0.38	31.6

These results indicate that the small amount of phytic acid phosphorus in high-grade flour is derived from the small particles of white endosperm intercellular tissue, which go through the silk-clothed dressing machines, and not from bran particles which are almost entirely absent in a well milled high-grade flour.

The tables below give the figures for various wheat mill products: Table VI for a mill working on the Simon's system, Table VII on

TABLE VI
ANALYSIS OF PRODUCTS FROM MILLING BY SYSTEM A

Product	Fiber	Phytic acid phosphorus	Total phosphorus	Percentage of total phosphorus as phytic acid phosphorus	Ash	Calcium
	%	%	%	%	%	%
Mill feed (100% whole meal)	1.76	0.203	0.35	57	1.56	0.046
72% extraction white flour	0.18	0.023	0.097	24	0.46	0.020
85% extraction wheat meal	0.91	0.11	0.189	59	0.90	0.041
Fine wheat feed	6.15	0.662	0.832	79.5	3.78	0.085
Bran	9.45	1.07	1.18	90	5.46	0.094
Germ	2.20	0.52	1.12	46	4.41	0.053
J. scalper tails	5.35	0.60	0.86	70	3.60	0.069
M. tails	2.15	0.46	0.54	85	2.70	0.037

TABLE VII
ANALYSIS OF PRODUCTS FROM MILLING BY SYSTEM B

Product	Fiber	Phytic acid phosphorus	Total phosphorus	Percentage of total phosphorus as phytic acid phosphorus	Ash	Calcium
	%	%	%	%	%	%
Mill feed (100% whole meal)	2.10	0.215	0.334	64	1.52	0.057
75% extraction white flour	0.25	0.053	0.117	45	0.53	0.025
85% extraction wheat meal	1.0	0.135	0.204	66	0.97	0.042
95% extraction wheat meal	1.62	0.162	0.271	60	1.29	0.055
Fine wheat feed	6.80	0.73	0.86	85	3.96	0.084
Bran	10.30	1.17	1.21	97	5.54	0.096
Germ	2.05	0.49	0.94	52	4.53	0.051
M. tails (equivalent)	3.55	0.715	0.742	96	3.39	0.080

another milling system. Both mill grists were similar, containing 65% Manitoban wheats, 15% Plate, 10% Australian, and 10% various wheats. The phytic acid phosphorus is in all cases estimated on the raw products; after fermentation and cooking in bread or similar products, varying degrees of hydrolysis (by phytase and other means) will take place, giving some inositol and inorganic phosphorus, varying with temperature, time of fermentation, and other factors.

The flour and tails from the various machines not represented in the tables all gave figures which, within the limits of experimental error, fall on the line of the graph shown in Figure 1. The figures show the

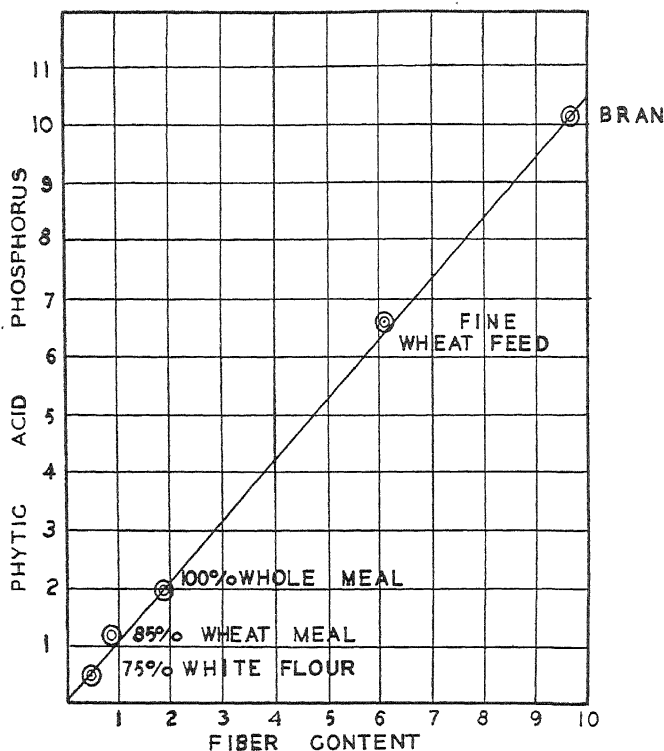


Fig. 1. Phytic acid phosphorus in relation to fiber content and percentage of extraction.

rapid increase in phytic acid phosphorus with the degree of extraction of the flour; thus 75% extraction flour has over double the amount of 72% flour. The lowest figure obtained was in C reduction flour, which gave 0.011% phytic acid phosphorus, which is about one-hundredth of the amount found in bran; this flour in pre-war days would have been classed as patent or high grade.

A preliminary investigation was made on the calcium salts of phytic acid. It was found that in the phytic acid extracted from bran, by the method of Harrison and Mellanby (1939) for oatmeal, the calcium salt obtained at pH values likely to exist in the digestive system

was a hexa-calcium salt, with a ratio of calcium to phosphorus of 40:31, but, as pointed out by the authors referred to, the phytic acid, in immobilizing a part of the calcium, also combines with varying amounts of magnesium, forming double salts. Accordingly no useful figures on the amount of calcium immobilized can very well be obtained by a theoretical study of the amount of phytic acid and calcium present in a product, as it is complicated by the amount of magnesium with which they will combine, and this, again, may depend upon various factors. The compositions of the calcium salts, produced at different pH values and dried at varying temperatures, are shown in Table VIII.

TABLE VIII
DATA ON CALCIUM SALTS

Solution in	pH	Temperature of drying	Calcium	Phosphorus	Probable composition
		<i>deg C</i>	\bar{c}_r	\bar{c}_p	
Dilute HCl	2.5	110	13.8	22.1	$\text{Ca}_3\text{P}_6 \dots$
50% acetic acid	1.2	20	15.3	17.9	$\text{Ca}_4\text{P}_6 \dots$
50% acetic acid	1.2	100	15.7	18.5	$\text{Ca}_4\text{P}_6 \dots$
50% acetic acid	1.2	110	16.5	19.1	$\text{Ca}_4\text{P}_6 \dots$
50% acetic acid	1.2	125	17.0	19.4	$\text{Ca}_4\text{P}_6 \dots$
50% acetic acid	1.2	150	18.2	21.3	$\text{Ca}_4\text{P}_6 \dots$
Acetic acid	3	110	19.8	18.8	$\text{Ca}_5\text{P}_6 \dots ?$
Acetic acid	4.5	110	23.3	19.2	Mixture of $\text{Ca}_4\text{P}_6 \dots$ and $\text{Ca}_6\text{P}_6 \dots ?$
Acetic acid	6.0	100	23.8	18.7	$\text{Ca}_6\text{P}_6 \dots$
Acetate	8.0	150	25.4	19.6	$\text{Ca}_6\text{P}_6 \dots$
Acetate	10.0	100	23.4	18.2	$\text{Ca}_6\text{P}_6 \dots$
Acetate	10.0	120	25.4	19.65	$\text{Ca}_6\text{P}_6 \dots$

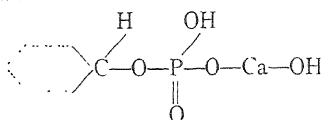
The tricalcium salt is very soluble in water; it is hygroscopic, but only slightly soluble in 90% alcohol. On boiling with water, or heating at 110°C., it appears to decompose into a higher calcium salt and phytic acid. The 1% cold water solution has a pH of 3.8.

The tetracalcium salt is produced in 50% acetic acid in which it is only slightly soluble; it is soluble in dilute acetic acid and water, but, like the tricalcium salt, gives an insoluble higher salt and phytic acid on boiling or heating dry.

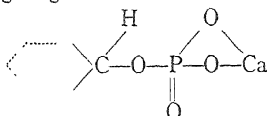
The pentacalcium salt could not be definitely isolated, but at pH3 the calcium salt precipitated agrees with this composition, although it may be a mixture of the tetra- and hexa-salts.

On approaching neutrality at pH 5.8 and on the alkaline side up to pH10, the hexacalcium salt is produced. This salt appears to be basic in character and its composition may be $\text{C}_6\text{H}_{13}\text{O}_{30}\text{P}_6\text{Ca}_6$ when dried at 100°C, but when heated to 150°C it loses three molecules of water, giving $\text{C}_6\text{H}_{12}\text{O}_{27}\text{P}_6\text{Ca}_6$, so it is possible that each of the six P atoms is

combined as below:



and when heated to higher temperatures than 100° loses one to six molecules of water, giving:



although other alternative formulae could explain this dehydration.

Experimental

The phytic acid phosphorus was estimated by the method detailed by McCance and Widdowson (1935), based on Brigg's original method. In making the first extraction with $0.5N$ HCl, the volume was made up to 100 ml. Thus, if 10 g of fine wheat feed was to be extracted, 91.5 ml of $0.5N$ HCl was added, giving a total volume of 100 ml of wheat-feed suspension. After two hours of shaking, 5 ml of the filtrate was used in the determination. This was proved to be nearly correct by removing 75 ml from the 100 ml and making up to 100 ml again with fresh $0.5N$ HCl, and extracting again for two hours. Then by determining the phytic acid in 20 ml of the filtrate instead of 5 ml as in the first extraction, almost identical figures were obtained, showing that the phytic acid is distributed evenly throughout the permeable mass, in the same proportion as in the surrounding liquid.

The second extraction had a tendency with some products to give a slightly higher result than the first—about 3% or 4%—indicating that the first extraction is not quite complete; thus a sample of bran, giving 1.17% phytic acid phosphorus on second extraction, gave 1.21%/4 phytic acid phosphorus; but differences such as this are within the experimental error of the method.

The total phosphorus and calcium were estimated in the usual manner, the calcium precipitated from dilute acetic acid as oxalate, titrated with $0.05N$ $KMnO_4$, and the phosphate after separation as phospho-molybdate precipitated and weighed as $Mg(NH_4)PO_4 \cdot 6H_2O$ on cindered glass Gooch, as described by Fales (1928).

Fiber was determined by the official method described in the Fertilisers and Feeding Stuffs Regulations (1928). In this connection it should be mentioned that flour and similar products with a fiber con-

tent below 0.5% give unsatisfactory results, as duplicate analyses may give nearly as wide variations as comparisons with a sample of bran containing 20 or more times as much fiber.

The preparation of a solution of sodium phytate from bran was made as described by Harrison and Mellanby (1939), for extraction from oatmeal: the solution was made slightly acid with acetic acid and boiled for two minutes to remove CO_2 , then rapidly cooled, and the pH adjusted by further addition of acetic acid or CO_2 -free NaOH. The 50% acetic acid solution contained CaCl_2 , and the precipitate was washed with 50% acetic acid till free from Cl. The tricalcium salt was precipitated from the dilute HCl solution by pouring this solution into nine or ten times its volume of 95% alcohol and washing the precipitate with 95% alcohol until free from Cl.

The hexacalcium salt was formed by adding calcium chloride or calcium acetate solution to the sodium phytate solution which had been boiled with dilute acetic acid for two minutes to remove CO_2 , before adding pure NaOH to the required pH. There was no evidence that a higher calcium salt than the hexa-salt could be produced even in a strongly alkaline solution, under these conditions; the precipitate formed contained slightly more calcium than the theoretical for the hexa-salt, but this was probably due to a small quantity of tricalcium phosphate resulting from a slight hydrolysis of phytic acid by boiling with dilute acetic acid.

The calcium and phosphorus were estimated in these salts by oxidation in a hard glass test tube with H_2SO_4 and HClO_4 as described by Harrison and Mellanby; the calcium was precipitated in dilute acetic acid as oxalate and phosphorus directly on the filtrate as $\text{Mg}(\text{NH}_4)\text{-PO}_4\cdot 6\text{H}_2\text{O}$.

Conclusions

In wheat mill commercial products the phytic acid phosphorus is proportional to the fiber content; but in certain constituents of those products, notably germ and parenchymatous cellular tissue of the endosperm, the phytic acid phosphorus is in a higher ratio to the fiber than in the pericarp tissue.

The calcium phytate likely to be produced in the alimentary tract is the hexacalcium salt.

Literature Cited

- Fales, H. A.
1928 Inorganic chemical analysis. Appleton-Century, New York.
Harrison, D. C., and Mellanby, E.
1939 Phytic acid and the rickets-producing action of cereals. *Biochem. J.* 33: 1660-1680.
McCance, R. A., and Widdowson, E. M.
1935 Phytin in human nutrition. *Biochem. J.* 29: 2694-2699.

THE ACTION OF AN OXIDIZING AGENT IN BREAD DOUGH MADE FROM PATENT FLOURS

J. C. BAKER, H. K. PARKER, and M. D. MIZE

Wallace & Tiernan Laboratories, Newark, New Jersey

(Read at the Annual Meeting, May 1941)

The action of oxidizing agents in altering the baking properties of dough has been the subject of intensive investigation by many workers. These investigations have been recently reviewed by Sullivan, Howe, Schmalz, and Astleford (1940). In their conclusions they suggest that the oxidation reaction causing baking improvement occurs in the protein of the flour but were unable to identify its character. Other workers, such as Jørgensen (1936) and Balls and Hale (1936), have stated that the improvement is due to the effect of the oxidizing agent on proteolytic enzymes or their activators in the flour, whereby proteolytic action is inhibited. However, Hale (1939) and Landis (1940) were able to find so little proteolytic enzyme in patent flours that much doubt is thrown on this hypothesis. The experiments of Read and Haas (1937) show that bromate used at commercial rates of application and commercial pH range did not inhibit proteases.

Bread doughs often soften with time. This softening is perhaps the reason many workers believe that proteolytic enzymes are involved in the reactions that affect bread quality. In order to study this softening of doughs we have modified the technique of Halton and Scott Blair (1937). Instead of extruding doughs and trying to obtain a test piece of fixed diameter, we have worked on a test piece of a definite weight and molded it to a definite length, letting it take whatever diameter was needed to accommodate the gases in the dough. The fixed weight of dough was always molded to the same length and placed on a mercury bath. Two ink marks were placed at a fixed distance apart on the dough surface, leaving sufficient dough outside of the marks for grasping it at each end. One end of the dough was attached to a spring scale while the other end was gripped by the fingers, stretched to a definite tension, and held for a definite length of time; then quickly released. Two readings were thus obtained—the total extension and the spring-back (elastic extension). Flow was calculated by recording the difference between the two readings. The spring-back and flow were recorded in all of our work. The sum of the two figures gave the total extension and the ratio of the two values could also be calculated.

It was noted that spring and flow change together in the same direction, but the changes of greatest magnitude were found in flow. In order to simplify a study of our results, flow has been the only

variable considered here. This is considered justifiable, as flow is largely responsible for the changes in ratio of viscosity to elasticity in doughs and certainly results in coalescence and breakage of dough bubbles in bread making.

In order to test the effects of oxidation and yeast upon bread dough, two series of doughs were made, one with and the other without yeast,—each series included doughs with moderate and heavy degrees of oxidation,¹ respectively. Table I shows a complete summary of the results obtained in these series of tests. In Figure 1 the results of flow have been charted.

TABLE I
EFFECT OF OXIDATION ON THE ELASTIC SPRING (S) AND VISCOUS
FLOW (F) OF DOUGHS, WITH AND WITHOUT YEAST

Doughs	Time after mixing					
	At once		30 min		90 min	
	S mm	F mm	S mm	F mm	S mm	F mm
YEASTLESS DOUGHS						
Unoxidized	33	18	44	23	70	41
“ + 5 ppm NaClO ₂	33	16	38	15	48	24
“ + 40 ppm NaClO ₂	36	18	31	6	28	4
DOUGHS + 2½% YEAST						
Unoxidized	34	20	32	12	34	18
“ + 5 ppm NaClO ₂	31	17	28	13	25	7
“ + 40 ppm NaClO ₂	39	14	24	5	16	2

All doughs irrespective of oxidation or presence of yeast showed substantially the same amount of flow when taken immediately from the mixer. The yeastless, unoxidized dough showed an increase in the amount of flow of the dough on standing, suggesting that some proteolytic action was taking place. The flow was largely prevented in the dough by a moderate degree of oxidation. This dough remained nearly unchanged with time, suggesting that such oxidation eliminated the proteolytic effects. A high degree of oxidation of the yeastless dough caused tightening of the dough with a marked decrease in flow, which progressively became less as time went on until the dough had very little flow at the end of the test period. This change in flow,

¹ Baker and Mize (1941) describe advantages of sodium chlorite as an oxidizing agent for research work.

opposite to that of the unoxidized dough, was not suggestive of a proteolytic effect.

Further, the proteolytic theory was not supported when yeasts were added to the doughs, for then the softening effects in the dough were almost entirely eliminated and the tightening effects produced by oxidation were intensified in every case. Apparently the yeast produced an effect very similar to that of the oxidizing agent. This suggested that both were reacting upon or affecting the same ingredient in the dough. One of the purposes of this work was to search for and investigate the nature and behavior of this ingredient.

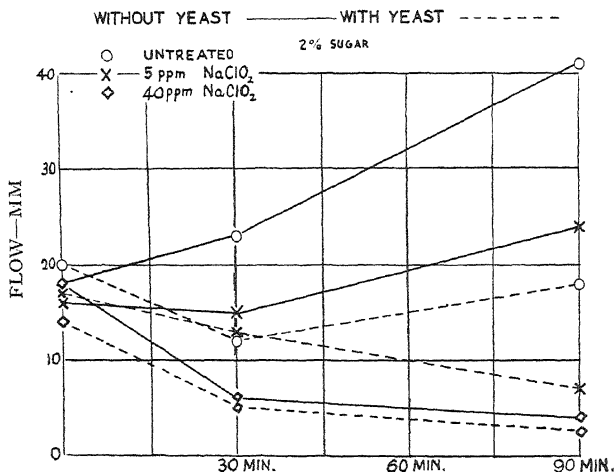


Fig. 1 Effect of oxidation on viscous flow of doughs

Bread was made from flour thoroughly extracted with petroleum ether and carbon tetrachloride. The "no-time" process of baking was used on these flours because the effects of oxidation are always more pronounced in this method of baking. In Figure 2, which shows the resulting bread, the first loaf was unoxidized. The second was made from a portion of the same dough to which 35 ppm of sodium chlorite was added. The very marked improvement in the baking qualities was evidence that the extraction of flour did not remove from the flour that property which resulted in baking improvement when the doughs were oxidized. Hence it appeared that this property is not due in any marked degree to the material extracted from flour by the fat solvents.² In order to determine in which portion of flour the reacting material

² Kosmin (1934) has shown interesting response to oxidizing agents in gluteins from extracted flours.

may be found, dough was separated by a gluten washing process into gluten, starch, "amyloextrin" (by centrifuging),³ and gluten wash solution. Each fraction was made up to one liter in $\frac{3}{4}\%$ salt solution, and the gluten fraction was dispersed in this volume of solution with a

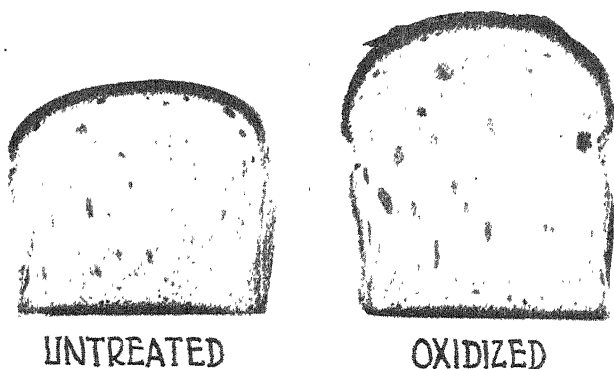


Fig. 2 The response of extracted flour to oxidation.

Waring mixer. To each solution the same amount of sodium chlorite was added and the solutions were permitted to stand 24 hours. The amount of sodium chlorite remaining was determined by titration. Table II gives the results of this experiment.

TABLE II
REACTION OF FLOUR CONSTITUENTS TO OXIDATION
(Each fraction from 100 g of flour reacted for 24 hours in 1 liter of $\frac{3}{4}\%$ NaCl solution with 9 mg NaClO_2)

Constituent	NaClO_2 remaining after 24 hours mg
Gluten	0.0
Starch	8.6
Amylodextrin	8.7
Solubles (gluten wash solution)	0.0
Blank—1 liter $\frac{3}{4}\%$ NaCl solution	9.0

The fractions containing gluten and gluten wash solution consumed all the sodium chlorite added. The fractions containing starch and amyloextrin consumed substantially none of the sodium chlorite in 24 hours, indicating clearly that the reactive material is to be found either in the gluten itself or in the gluten wash solution. We did not further investigate the relation of starch and "amyloextrin" to this

³ This material was described by Sandstedt, Jolitz and Blish (1939), page 781.

problem. The findings with reference to the lipoid and starch portions of flour are in agreement with results reported by Sullivan, Howe, Schmalz, and Astleford (1940).

Preliminary observations of the action of oxidizing agents upon gluten showed very marked changes in gluten properties. An apparatus was therefore built to study gluten physical properties.

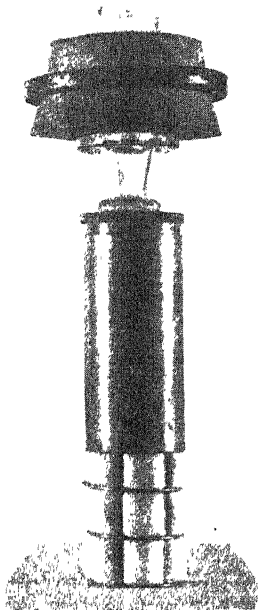


Fig. 3. Gluten testing device.

Figure 3 shows the device, which consists of a cylindrical wire cage attached to a tube in which the operation of a plunger is actuated by air pressure. The gluten is placed so that it fills the cage and extends into the cylinder. The plunger pushing down forces it to fill the space and bulge out through the large meshes in the wire screen. Upon release of the pressure, the gluten springs back to relieve the strain. The downward and upward motions of the plunger are measured and the difference between the two measurements indicates the flow or viscosity of the gluten. The value of this device lies in the fact that the gluten is confined to a definite shape, which makes it possible to secure reliable and reproducible measurements. Table III shows the physical properties of a series of glutes prepared from one flour under different

conditions. The results are expressed in units of the plunger scale as "spring" and "flow." There is no numerical relationship between these readings on gluten and those above on dough as the two instruments involved give results which cannot be translated from one to the other.

TABLE III
EFFECT OF DOUGH ENVIRONMENT ON ELASTIC SPRING (S) AND PLASTIC FLOW (F) OF GLUTEN

Origin of gluten	S	F
1. Yeastless dough washed in 3 liters of $\frac{3}{4}\%$ NaCl solution after mixing	1.76	0.64
2. Gluten No. 1 allowed to stand 4 hours	1.80	0.55
3. Dough of No. 1 stood 4 hours before washing	1.57	0.57
4. Same dough containing $2\frac{1}{2}\%$ yeast stood 4 hours before washing	1.18	0.16
5. Gluten No. 1 washed in 3 additional 3-liter solutions	1.26	0.16
6. Gluten No. 5 worked in 40 ppm NaClO ₂ in last solution	1.17	0.14
7. Gluten No. 1 worked in 200 ml of $\frac{3}{4}\%$ salt solution + 40 ppm NaClO ₂	1.11	0.22
8. Gluten No. 1 worked in 200 ml of $\frac{3}{4}\%$ salt solution only	1.34	0.51
9. Gluten No. 1 worked in 200 ml of $\frac{3}{4}\%$ salt solution containing 200 ppm papain and then stood one hour	1.70	1.12

Note: Gluten No. 1 washed 20 minutes in 3-liter solution, then two additional 200 ml 5-minute washes (all other washings above were 20 minutes each).

In the gluten experiments reported in this paper all operations have been conducted in $\frac{3}{4}\%$ salt solution prepared with boiled distilled water saturated with carbon dioxide. A current of carbon dioxide was also bubbled through the solution during the experiments. All doughs had been mixed in the absence of air in a carbon dioxide atmosphere, unless otherwise stated. Throughout this paper one flour only has been used—a Southwestern patent containing 11.25% protein and 0.40% ash.

It is to be noted in Table III that the changes in spring are proportionally smaller than are the changes in flow. In order to simplify the consideration of the effects produced on the gluten, flow only will be discussed.

Gluten No. 1 was the basic gluten with which all other glutes considered in Table III were compared. It had the greatest flow of any of the glutes. *Gluten No. 2*, after standing four hours, showed a small amount of tightening and loss of flow. This is in contrast to the softening sometimes observed in doughs. *Gluten No. 3* showed that if the dough itself stood four hours before washing the result was substantially the same as in the instance where the washed gluten stood four hours. If the theory that proteolytic enzymes soften gluten in a dough is correct, it is hardly conceivable that this should be the case, for the washing of a gluten must remove much of the enzymatic material.

Gluten No. 4 was similar to No. 3 with the exception that 2½% yeast acted on the dough for the four hours. The flow properties of the gluten had now almost entirely disappeared because of the fermentation. This indicates that the changes produced in dough by yeast, as shown in Table I, are found in the gluten. Tightening of the dough was accompanied by corresponding tightening of the gluten, indicating that changes in dough properties caused by yeast are also to be found in the gluten itself. *Gluten No. 5* was a portion of No. 1 washed in three additional three-liter portions of solution. It may be noted that this gluten, merely by washing, exhibited the same properties as were found in the gluten from the dough which had been acted upon by yeast. This indicates that the material whose removal is responsible for the tightening of the gluten is soluble in the solution and that by washing it out a reaction similar to that with yeast is obtained.

Gluten No. 6 was prepared exactly like No. 5 except that the last three-liter portion of wash water contained 40 ppm of sodium chlorite. The manipulation of this gluten in the oxidizing solution has produced only a very slight tightening. *Gluten No. 7* was produced by subjecting another portion of No. 1 to a similar treatment in a small volume of wash water containing 40 ppm of sodium chlorite. It is to be noted that the soft gluten now became a very tight, compact, rubbery mass, similar to the glutens obtained either by yeast or by extreme washing. In order to show that this effect was not due to the small amount of water in which the gluten was manipulated, *gluten No. 8* was a similar gluten worked in 200 ml of solution containing no sodium chlorite. Here only a slight tightening of the gluten occurred. Finally, gluten No. 1 was washed in a similar 200-ml portion of solution containing 200 ppm of commercial papain, as shown by *gluten No. 9*. This gluten exhibited a marked softening from the treatment, indicating that proteolytic enzymes, when present, soften gluten, giving a change in properties opposite to that obtained from all of the above treatments. This indicated that the material in gluten causing its response to oxidizing agents is not proteolytic in nature and suggested a further study of the water solubles.

In an attempt to further extract the solubles from gluten, it was dispersed in a "Waring Blendor"⁴ and the dispersed gluten suspension poured into centrifuge tubes and whirled. The gluten came together either at the top or at the bottom of the tube, or both, and was collected as firm, packed gluten, appearing substantially the same as before it went into the blender. However, this gluten was unusually tough and, though extensible, it showed the least flow of any gluten prepared.

⁴ Freilich (1941) described this apparatus and its applicability to flour suspension problems. Our modification maintains the material under CO₂ during preparations.

This dispersed gluten was then redispersed and collected, and the same operation repeated a total of six times. The resulting gluten was similar to that obtained from the first dispersion. The successive solutions in which this gluten was dispersed were analyzed for nitrogen, as shown in Table IV.

TABLE IV
SOLUBLE PROTEIN FROM SUCCESSIVE WASHINGS OF GLUTEN BY
HAND AND BY MECHANICAL DISPERSION
(270 g flour washed in oxygen-free CO₂-saturated $\frac{3}{4}$ % salt solution)

Wash solution analyzed	Protein in total wash solution	Concentration of protein in wash solution
		<i>g per l</i>
First 1.8 liter wash solution	4.457	2.48
After three more 1.8-liter 20-minute workings	0.235	0.13
After dispersion in 375 ml of solution	0.582	1.55
After 2nd dispersion	0.354	0.94
After 3rd dispersion	0.268	0.72
After 4th dispersion	0.222	0.59
After 5th dispersion	0.184	0.49
After 6th dispersion	0.178	0.48

The four gluten wash solutions each amounted to 1800 ml. The dispersions were each in 375 ml of solution and were made with 90 grams of wet gluten.

It is to be noted that there is a large increase in the solubility of the protein upon dispersion. By breaking up the gluten into an extremely fine suspension, as obtained in a Waring mixer, a large amount of protein was obtained which was so soluble that it could not be centrifuged or filtered out. On successive dispersions of the remaining gluten, decreasing amounts of soluble nitrogenous material were found until on the fifth and sixth dispersions nearly a constant solubility was found, suggesting that the soluble protein was not largely a component of gluten itself. The first dispersate protein fraction exhibits the characteristic properties of a proteose. It was very slowly coagulated with heat and required saturation with ammonium sulfate to obtain complete precipitation. Thus it seems that this protein is different from the gluten itself. The presence of this dispersible fraction in gluten, which can be removed by very violent mechanical means from the body of the gluten, suggests that this is a material which may be responsible for the slippage in the gluten before dispersion.

In order to study further the dispersible fraction of gluten, certain glutens reported in Table III were dispersed and the amount of the dispersible protein measured in the first dispersate liquor as shown in Table V.

TABLE V
 PROTEINS SOLUBLE BY DISPERSING GLUTENS IN WARING BLENDOR
 (Soluble protein in grams per liter)

Preparation of gluten	Gluten wash solution	First dispersate liquor from gluten obtained by	
		Ordinary washing	Extreme washing
Gluten from unfermented dough	2.32	1.78	1.55
Gluten from 4-hour fermented dough	2.24	1.45	1.15
Gluten from 4-hour unfermented dough	2.39	1.49	1.36

Note that ordinary gluten carries more dispersible nitrogenous material than gluten obtained by thorough washing or by fermentation, suggesting that these two operations have each removed some of the dispersible fraction and thus rendered the glutens less likely to flow when tested as shown in Table III.

In order to find the response of gluten to the solubles in flour, a concentrated gluten wash water was prepared. Gluten was washed from a dough in an equal weight of $\frac{3}{4}\%$ salt solution. The starch and "amylodextrin" were centrifuged from the wash solution, which was then reused to continue the washing of the gluten, thereby obtaining substantially complete removal of starch and "amylodextrin." Gluten prepared in this manner exhibited unusual properties, some of which are shown in Table VI.

TABLE VI
 GLUTEN WASHED FROM A DOUGH IN AN EQUAL WEIGHT OF $\frac{3}{4}\%$ SALT SOLUTION AND
 REWASHED IN SAME SOLUTION CENTRIFUGED TO REMOVE STARCH
 AND AMYLODEXTRIN

	Spring	Flow
Gluten as prepared	3.13	2.13
Same gluten washed in an equal volume of $\frac{3}{4}\%$ salt solution	1.26	0.44
Same gluten worked in original centrifuged wash solution +0.1% NaClO ₂	1.87	0.21

It exhibited approximately three times the flow of any of the glutens previously prepared. However, when washed in one small equal volume of salt solution alone, its flow dropped enormously, giving a gluten with less flow than one prepared by the ordinary method. When this gluten was similarly washed in water containing sodium chlorite, it tightened and nearly all of its flow properties disappeared. Apparently one can dissolve out the property that is responsible for flow in gluten or one can oxidize it so that its effects disappear. These effects can be produced and measured in so short a time interval that interpretation of results by enzymatic explanation seems improbable.

The remarkable change in gluten properties produced by washing in a small volume of salt solution led us to study the properties of a gluten which was merely manipulated in a smaller volume of solution for increasing lengths of time. It then became apparent that merely working a gluten in a solution without changing the water at all, causes it to tighten almost as much as does extreme washing, yeast, or oxidation. It is now apparent that gluten is composed of materials which can be entangled or enmeshed by mechanical working so that the molecules do not slip by one another. This suggests that gluten may be composed of molecules that are shaped like coiled springs. If one would take a mass of small coil springs and work them together, changes in the properties of the mass similar to that observed in gluten would be obtained. The more they are worked the harder they are to separate. The dispersible fraction which is in gluten may interfere with the intermeshing of the coils and may also lubricate the molecules so they readily slip apart. In order to study this hypothesis a series of glutes were worked in the strong gluten wash solutions. The results are reported in Table VII.

TABLE VII
EFFECT OF WORKING GLUTENS IN CONCENTRATED
CENTRIFUGED GLUTEN WASH SOLUTIONS

(S = spring; F = flow)

Type of gluten	Unworked		Worked	
	S	F	S	F
Gluten by 5 dispersions	1.17	0.13	1.16	0.09
Gluten by thorough washing	1.36	0.18	1.23	0.29
Gluten from fermented dough	1.27	0.15	1.15	0.14
Gluten by ordinary washing	1.46	0.54	1.53	0.70

The redispersed gluten which was substantially free from dispersible material gave a noticeable tightening upon being worked in this strong gluten wash solution. The fermented gluten did not change. The ordinary gluten and the thoroughly washed gluten showed softening. This suggests that the gluten wash waters work into the meshes of the gluten and cause slippage only where dispersible material is present.

The effect of an oxidizing agent on the physical properties of the same four glutes was studied, as shown in Table VIII.

Substantially no effect upon the fluid properties of these glutes was obtained by manipulating them for 20 minutes in 40 ppm sodium chlorite, except in the case of the gluten produced with ordinary washing. The gluten obtained by thorough washing did not react, thus

TABLE VIII
EFFECT OF OXIDATION ON PHYSICAL PROPERTIES OF GLUTEN

(S = spring; F = flow)

Type of gluten	Unoxidized		Oxidized	
	S	F	S	F
1. Gluten by 5 dispersions	1.38	0.11	1.34	0.12
2. Gluten by thorough washing	1.26	0.16	1.17	0.14
3. Gluten from fermented dough	1.18	0.15	1.15	0.15
4. Gluten by ordinary washing	1.76	0.64	1.11	0.22

suggesting that the material in gluten which reacts to oxidizing agents is soluble in water or removable by yeast.

Summary

The method of testing doughs developed by Halton and Scott Blair (1937) has been modified to simplify the sampling technique, making it possible to work on fermented as well as unfermented doughs and compare the results.

The property of flow, which is the most significant characteristic of doughs, as measured by this method, shows that unyeasted doughs from patent flour containing sugar may soften with time. These doughs upon addition of yeast or of an oxidizing agent lose the property of softening and if treated more heavily they tighten progressively.

Experiments indicate that the reaction of doughs to oxidizing agents is not concerned in any marked degree with the starch, "amylo-dextrin," or fat portion of the dough, but is located in either the gluten or the water-soluble portion of the dough.

A device was built to test the same characteristics of gluten as were tested on doughs by the apparatus of Halton and Scott Blair. Tests indicate that glutes prepared from unfermented doughs containing no yeast react to oxidizing agents. When prepared from fermenting doughs containing yeast, tight glutes which do not react are obtained. This indicates that the response of gluten to these treatments is similar to that of doughs.

Upon subjecting glutes to thorough washing conditions it was observed that the property of flow could be largely removed from the gluten, indicating that this property is associated with water-soluble constituents.

Glutes subjected to the action of papain undergo a marked progressive softening, opposite to the effects of oxidation. Changes in gluten similar to oxidation are produced by extensive washing.

Water solubles of gluten were further extracted by dispersing mechanically in $\frac{3}{4}\%$ salt solution with a Waring Blendor and the gluten collected by centrifuging. When gluten which had been subjected to thorough washing conditions was thus dispersed, a relatively large amount of protein material was rendered soluble. Further, decreasing quantities could be removed on successive dispersions until finally the amount of soluble protein removed by each dispersion reached a constant value, indicating that all of a readily dispersible type of material has been removed and only a true gluten component was now dissolving. This dispersible material showed many of the properties of a proteose. Glutens that had been subjected to washing or to fermentation showed less of this dispersible fraction than ordinary glutens. Glutens receiving fermentation showed less than the washed gluten, indicating that fermentation had removed some of this material, thus possibly explaining some of the tightening effect on dough exhibited by yeast.

Glutens were manipulated in the most concentrated "gluten wash solution" we were able to prepare. The redispersed gluten tightened as a result of handling, just as any gluten ordinarily will when manipulated in salt water. The gluten from yeast fermentation showed no change from the handling. Rewashed or ordinary gluten showed marked softening from this treatment, indicating that the dispersible fraction left in the gluten was softened by the ingredients of the strong gluten wash solution and rendered more fluid so that the meshing of the gluten molecules became less firm and slippage occurred.

The same four glutens were manipulated in salt solution containing sodium chlorite. Substantially no change occurred in any of the glutens except the one subjected to ordinary washing. This gluten exhibited a marked tightening, much greater than would have occurred had not the oxidizing agent been present. This evidence suggests that the material in gluten which reacts to the oxidizing agent is soluble in salt water and can be washed from the gluten by sufficient treatment, and it indicates that the reactive material is found in the more readily dispersible fraction.

Literature Cited

- Baker, J. C., and Mize, M. D.
1941 The origin of the gas cell in bread dough. *Cereal Chem.* **18**: 19-34.
Balls, A. K., and Hale, W. S.
1936 Proteolytic enzymes of flour. *Cereal Chem.* **13**: 54-60.
Freilich, J.
1941 A method for the determination of reducing matter in flour. *Cereal Chem.* **18**: 129-137.
Hale, W. S.
1939 The proteinase in wheat flour. *Cereal Chem.* **16**: 695-702.

- Halton, P., and Scott Blair, G. W.
1937 A study of some physical properties of flour doughs in relation to their bread-making qualities. *Cereal Chem.* **14**: 201-219.
- Jørgensen, H.
1936 On the existence of powerful but latent proteolytic enzymes in wheat flour. *Cereal Chem.* **13**: 346-355.
- Kosmin, Natalie P.
1934 Kleberqualität und einige Faktoren, die diese beeinflussen. *Mühlenlab.* **4**: 110-115.
- Landis, Q.
1940 Polariscopic determination of proteolytic activity. *Cereal Chem.* **17**: 468-472.
- Read, J. W., and Haas, L. W.
1937 Studies on the baking quality of flour as affected by certain enzyme actions. IV. Potassium bromate and enzyme activity. *Cereal Chem.* **14**: 752-765.
- Sandstedt, R. M., Jolitz, C. E., and Blish, M. J.
1939 Starch in relation to some baking properties of flour. *Cereal Chem.* **16**: 780-792.
- Sullivan, B., Howe, M., Schmalz, F. D., and Astleford, G. R.
1940 The action of oxidizing and reducing agents on flour. *Cereal Chem.* **17**: 507-528.
-

AN IMPROVED METHOD FOR THE VOLUMETRIC DETERMINATION OF SODIUM CHLORIDE IN BREAD

ROY IRVIN

Red Star Yeast & Products Co., Milwaukee, Wisconsin

(Read at the Annual Meeting, May 1941)

Investigation showed that the official method for the determination of sodium chloride in bread gave low and variable results, less than 70% of the salt being recovered when the method as outlined in *Cereal Laboratory Methods* (A. A. C. C., 3rd ed., 1935) was followed. As it was suspected that chloride was being lost through sublimation during ignition, the addition of various alkaline substances to bread crumb prior to ashing was investigated. Reports of studies by others also indicated loss of chloride during ashing of bread crumb and similar substances. Hoffmann, Schweitzer, and Dalby (1940) found that sodium hydroxide prevented the loss of iron through sublimation of ferric chloride during the burning of bread crumb and Kent-Jones (1927) made use of alcoholic sodium hydroxide in the recovery of chloride from flour fat.

In this work the procedure as given in *Official and Tentative Methods of Analysis* (A. O. A. C., 4th ed., 1935) for the determination of chloride in plant materials was taken as a basis for an improvement of the present official method for salt in bread. According to the A. O. A. C. test, sodium carbonate is employed to prevent chloride escape.

Experimental

Preliminary analyses indicated conclusively that the ashing of bread crumb without a fixative agent for chloride would yield low salt values, but that good recovery was possible when the crumb was treated with sodium carbonate or sodium hydroxide before ashing. Less favorable results were obtained with zinc oxide, calcium carbonate, magnesium oxide, and magnesium acetate (alcoholic solution). Several analyses of dried bread crumb, as well as of flour containing added salt, showed an average chloride loss of 30% to 35% when no alkaline substance was added to the samples before ashing, and a 10% to 15% loss when the samples were first treated with calcium or magnesium compounds. In case of the alkaline earths, a single ashing of crumb usually sufficed, but with the sodium compounds it was necessary to leach the charred sample with water or dilute nitric acid before the carbon could be completely burned.

A few determinations of salt in bread were also made by adding three successive portions of 5 ml of 4% alcoholic potash to 3-g samples of crumb during ignition. This was followed by a single filtration of the ash. Although the recovery of chloride by this method was fairly good—being about 97% to 98% of the calculated value—some loss of material occurred through spattering, and only in the case of bread samples made from a no-salt formula was a white ash obtained. When the crumb contained a normal amount of salt, the ash fused badly with the alcoholic potash procedure and showed particles of unburned carbon. The disadvantages of this method might be overcome through further investigation, thus making possible the elimination of the double ashing found necessary with sodium carbonate.

To expedite the analysis of ash of the bread crumb, Caldwell's modification of the Volhard volumetric method for chloride was chosen as a satisfactory procedure. The method as used in this investigation is described by Kolthoff and Sandell (1936). The Caldwell method, which does not require removal of the precipitated silver chloride before back titration with thiocyanate as in the usual Volhard procedure, was found by trial to be sufficiently accurate for the purpose in hand.

Bread for the experimental work was made in the laboratory from two types of formulas, one containing 5% sugar, 3% shortening, and 3% yeast, the other containing 5% sugar, 3% shortening, 5% skim-milk solids, 0.5% malt syrup, 0.3% yeast food, and 3% yeast. The salt content was varied from 0 to 2.25%. The ingredients were carefully weighed for each loaf, all of the dough was used, and the crumb from the dried loaf was quantitatively recovered, thus making it possible to calculate the added salt content of the dried crumb. Chloride blanks for the nonsalt materials were obtained by analysis of

bread made without salt. In Table I, samples 1 and 2 were derived from bread made according to the lean formula and samples 3, 4, 5, and 6 were from bread prepared from the enriched formula. In the case of the no-salt doughs, fermentation time was shortened to prevent excessive loss of materials through rapid yeast action.

TABLE I
SUMMARIZED RESULTS OF SALT DETERMINATION IN BREAD—DRY BASIS

	Sample 1 (blank)	Sample 2	Sample 3 (blank)	Sample 4	Sample 5	Sample 6
	%	%	%	%	%	%
Total percent found	0.116	2.15	0.273	1.78	2.24	2.52
	0.114	2.15	0.283	1.77	2.26	2.52
	0.114	2.14	0.283	—	2.26	—
	—	2.16	—	—	—	—
	—	2.15	—	—	—	—
	—	2.18	—	—	—	—
Average	0.115	2.16	0.280	1.78	2.25	2.52
Net percent, average	—	2.05	—	1.50	1.97	2.24
Calculated percent	—	2.090	—	1.506	1.995	2.241

Prior to analytical work, the glassware employed was calibrated and during the determinations corrections were made for variations in the temperature of standard solutions. Strongly heated sodium chloride of the highest grade obtainable was used as an ultimate standard. Its purity was checked against fused silver nitrate by both the gravimetric and the Caldwell methods.

The modified procedure for the determination of salt in bread is as follows:

Reagents:

1. Silver nitrate. Adjust to 0.05*N* strength by standardizing against 0.05*N* NaCl solution containing 2.923 g pure NaCl per liter.
2. Potassium thiocyanate. Adjust to 0.05*N* by titrating against 0.05*N* AgNO₃.
3. Ferric indicator. Saturated solution of ferric ammonium alum.
4. Dilute nitric acid. Dilute the usual pure acid with $\frac{1}{4}$ volume H₂O. Boil until colorless and then add H₂O to make a 1 + 4 dilution of the acid.
5. Nitrobenzene (cp).
6. Sodium carbonate, 5.0% solution.

Determination: Prepare the samples as directed in Chapter VI, sec. 1, page 83, *Cereal Laboratory Methods*, and determine the moisture in

the air-dried crumb as directed in Chapter VI, sec. 2, page 83. (The air-dried crumb may be first dried as directed in (a) or (b) on page 29, *Cereal Laboratory Methods*, and then weighed.) Weigh 3 g of the air-dried (or completely dried crumb) in a porcelain or platinum crucible and thoroughly wet with 10 ml of 5% Na_2CO_3 solution. Dry at $120^\circ\text{--}130^\circ\text{C}$ for about one hour to remove excess moisture, then thoroughly char at a dull-red heat (2–4 hours' ignition). After the crucible is cool, cover the char with water, let stand a few minutes in a warm place, cautiously acidify with 1 + 4 HNO_3 and filter. The filter is well washed with water and small amounts of dilute acid, a total of 15–16 ml of 1 + 4 HNO_3 being used for neutralizing and washing. Place the paper and char back in the crucible, partially dry and then ignite to a white ash at a dull-red heat (30–45 minutes required). Solution of ash, acidification, and filtration are carried out as before except that only 2–3 ml of 1 + 4 HNO_3 is required. The second filtration is added directly to the first. Combined filtrates and washings should not amount to more than about 125 ml. Add 25 ml of 0.05N AgNO_3 , 3 ml of nitrobenzene, and 1 ml of ferric indicator in the order given. Shake thoroughly for about 30 seconds or more and titrate the excess AgNO_3 with 0.05N KCNS solution to a faint reddish-brown tint that does not fade in 3 or 4 minutes. As the true end point is approached a false end point may occur which disappears upon vigorous shaking.

Calculation:

$$\frac{(\text{ml AgNO}_3 \text{ sol} - \text{ml KCNS sol}) \times 0.002923 \times 100}{\text{weight sample}} = \text{percent salt.}$$

Discussion of Results

The order of accuracy obtainable by the revised procedure is indicated by the results given in Table I. It will be recalled that sample No. 1 constituted a blank for the nonsalt materials in sample No. 2, while No. 3 was a blank for the remaining samples (Nos. 4, 5, and 6).

Portions of the same six samples of bread crumb were submitted to three collaborating laboratories for check determinations by the revised method. The results reported by the collaborators are shown in Table II.

While the comparative results found by the different laboratories do not agree as closely as could be desired, the method as described can be considered a distinct improvement over the official procedure for the determination of salt in bread.

TABLE II

RESULTS REPORTED BY COLLABORATORS: NET PERCENT SALT FOUND—DRY BASIS

Sample	Collaborators			Author	Calculated values
	A	B	C		
	%	%	%	%	%
2	2.04	1.91	2.01	2.05	2.090
4	1.52	1.34	1.46	1.50	1.506
5	1.96	1.88	1.94	1.97	1.995
6	2.27	1.95	2.21	2.24	2.241
BLANK VALUES					
1	0.150	0.180	0.13	0.115	—
3	0.325	0.260	0.28	0.280	—

Conclusions

The official method for the determination of salt in bread gives low results due to loss of chloride during ashing.

Substantially complete recovery of chloride from bread crumb can be obtained by the addition of sodium carbonate prior to ashing.

Caldwell's modification of the Volhard procedure for chloride is rapid and sufficiently accurate for the analysis of the ash of bread.

It is suggested that the substitution of alcoholic potash for sodium carbonate in this determination be further investigated.

Acknowledgment

The author wishes to express his thanks to collaborators C. N. Frey, E. L. Von Eschen, and F. A. Collatz for the generous assistance rendered by them in this investigation.

Literature Cited

- American Association of Cereal Chemists
 1935 Cereal laboratory methods (3rd ed.).
 Association of Official Agricultural Chemists
 1935 Official and tentative methods of analysis (4th ed.). Washington, D. C.
 Hoffmann, C., Schweitzer, T. R., and Dalby, G.
 1940 Iron content of bread and bread ingredients. Ind. Eng. Chem. (Anal. Ed.) 12: 454-455.
 Kent-Jones, D. W.
 1927 Modern cereal chemistry (2nd ed.). Northern Publishing Co., Liverpool.
 Kolthoff, I. M., and Sandell, E. B.
 1936 Textbook of quantitative analysis. Macmillan Co., New York.

REPORT OF THE 1940-41 COMMITTEE ON METHODS OF TESTING CAKE FLOUR

J. W. MONTZHEIMER, *Chairman*

Centennial Flouring Mills Co., Spokane, Washington

(Read at the Annual Meeting, May 1941)

The object of this year's committee on methods of testing cake flour was to determine the value of the A. A. C. C. tentative cake test and the sugar and shortening supplements described in the book of methods for determining the value of cake flour in commercial formulas.

TABLE I
ANALYSIS ON CAKE FLOURS¹

Sample	Ash	Protein	Moisture	pH	Viscosity
	%	%	%		° MacM
W	0.361	7.0	12.5	4.9	32
X	0.36	8.4	12.4	5.2	68
Y	0.405	8.5	12.6	5.3	70

¹ Analysis by V. H. Morris, Federal Soft Wheat Laboratory, Wooster, Ohio.

Three flours from widely separated sections of the country were chosen (Table I). Samples of these flours were submitted to committee members, by whom they were baked according to the A. A. C. C. formula with supplements and scored by the official score card for cake together with the stop grades as proposed by the 1939-40 committee. For average scores on flour submitted, see Table II. Ratings of the flour by committee members are shown in Table III. The same flour samples baked in regular commercial cake formulas and judged by bakers' service men are rated in Table IV.

TABLE II
AVERAGE OF COLLABORATORS' SCORES

Formula and supplement	X			Y			W		
	Av. volume	Av. score	Av. grade	Av. volume	Av. score	Av. grade	Av. volume	Av. score	Av. grade
	cc			cc			cc		
A. A. C. C.	730	81	Good	750	77	Fair	730	82	Good
Sugar 10%	713	79	Good	754	79	Fair	708	80	Good
Sugar 20%	736	79	Good	750	72	Fair	729	74	Fair
Sugar 30%	712	73	Poor	744	68	Poor	715	64	Poor
Shortening 25%	751	83	Good	781	79	Good	761	83	Good
Shortening 50%	742	83	Good	759	77	Good	719	83	Good
Shortening 75%	702	79	Fair	719	77	Fair	662	86	Good
Av. of all tests	726	79.5	—	751	75.5	—	718	80	—

TABLE III
RATINGS ACCORDING TO A. A. C. C. FORMULA

Committee member	1st	2nd	3rd	Comments
Armstrong .	W	X	Y	Y increased in volume with added sugar and shortening. Recommended for rich formulas.
Haley	X	W	Y	X for all-round cake purposes. W for cookies.
Mitchell .	Y	X	W	Y best volume. X best symmetry.
Montzheimer.	W	X	Y	X and W about equal. Y coarse grain and poor color.
Stokes	W	X	Y	Y best for pound cakes. X for medium rich sugar and light loaf. W is best for sponges and angel cakes.
Wade	W	X	Y	Flours all good.

TABLE IV
RATINGS BY COMMERCIAL FORMULAS

	1st	2nd	3rd
White layer	W	Y	X
Yellow layer	W	X	Y
Devil's food	W	X	Y
Pound cake	Y	X	W
Sponge cake	X	W	Y
Angel cake	X	W	Y

The committee feels that the following conclusions may be drawn from this year's work:

1. It would appear that W is best suited for layer and devil's food cake, Y for pound cake, and X for sponge and angel cake.

2. The A. A. C. C. formula with supplements is valuable in predicting the behavior of cake flours in commercial formulas containing shortening.

3. The A. A. C. C. formula does not indicate the suitability of a flour for angel or sponge cakes.

4. Bakers and commercial men rate volume the most important in judging cakes. It has been suggested that our present rating system lays too much importance on cake appearance, color, grain, and crust, and not enough value is placed on volume.

The following suggestions are recommended for the new committee: (1) That this year's project be repeated, except that changes in sugar and shortening should be accompanied by a balance of the other ingredients. This year's committee felt that the present supplements spoiled the cake, because the ingredient ratio was wrong. (2) Further investigation of mixing should be undertaken, including a study of the

right time for adding leavening agents and the use of specific volume as a method of determining the final mixing time. (3) Collaborative studies should be made at various altitudes to check the work done by Mr. Barmore, for correction of the baking powder in A. A. C. C. formulas at various altitudes. (4) This year's committee believes that a great deal of worth-while work could be carried out by local cake committees in the various sections.

Acknowledgment

This year's work was carried out by the following committee members: Donald Wade, Lowell Armstrong, William Haley, W. E. Stokes, R. W. Mitchell, and K. Rourbaugh.

GRANULATION AS A FACTOR IN CAKE FLOUR QUALITY

W. H. HANSON

Commercial Milling Company, Detroit, Michigan

(Read at the Annual Meeting, May 1941)

Researches by capable workers such as Alsberg, Mayer, Ling, Katz and numerous others have added greatly to our knowledge of the wheat starches. The work of the cereal chemists, particularly those who are located within the soft winter wheat area, has accomplished much in evaluating the importance of tests such as hydrogen-ion concentration, viscosity, diastatic activity, and flour granulation when these factors are applied to actual bake shop performance.

A review of the literature on flour granulation indicates that this subject has received a great deal of consideration. Alsberg and Griffing (1925) showed the effect of overgrinding on hard winter and spring wheat varieties, and the subsequent effect on loaf volume. Woodruff and Nicoli (1931) summarized the importance of starch gels as affecting the flour quality. Sandstedt, Jolitz, and Blish (1939) indicated the importance of starch in relation to some baking properties of flour, and state that "certain undesirable baking characteristics of some exceedingly hard wheat starches are due to damage to the starch in milling." Jones (1940) summarized the importance of mechanically damaged starch in milling, and its effect upon the diastatic activity of flour. Alexander (1939) suggested a method for determining the granulation of flours in connection with the usual laboratory routine tests which are made.

Hastings (1938) states that "the size of the starch cells of wheat is apparently influenced to a large degree by the variety, growing and ripening conditions, and the protein strength." The size of the starch cell of soft winter wheat is therefore obviously important, in that a

finely milled flour seems better adapted for cake manufacture. It is our opinion that the granulation of a flour may change from one crop to the next, depending upon the growing and ripening season of the grain. If such changes are manifested, it is necessary to make the proper changes in milling to arrive at a satisfactory degree of fineness in granulation comparable to the previous year.

Our tests have indicated that the size of the flour particle is not dependent upon the use of exceedingly fine silk bolting cloths in the flow of a mill. A proper balance of the mill stocks in the bolters and purifiers and selective grinding operations seem more important to the resulting flour. Controlled breaking of the mill stocks is very essential, and should be carefully checked in order to maintain the proper balance. Small sifters are now being used in many mills to good advantage as a means of checking hourly any change which might occur in the grinding operation.

Jones (1940) states that "all flours contain a certain proportion of their starch granules mechanically damaged as a result of the milling process." It seems impossible to mill soft winter wheat flours without dislodging starch granules from the matrix surrounding each particle. The presence of "ghosts" in flour is very important, in that ruptured starch granules should be avoided as much as possible in the grinding operation. While a fine granulation seems to be very desirable, the grinding must not be carried to the extent of mechanical rupture of the starch granule, and a subsequent weakening of the gel strength in cake batters.

Alsberg and Griffing (1925) state that "the strength of the gel is dependent upon the size to which the granules swell, and the volume which they occupy as compared to the total volume in which they are suspended." Tests which have been made show quite conclusively that the gel strength is associated with the size and number of starch granules present in the batter emulsion. Flours which are milled from the same wheats but varying 5% or more in granulation¹ show decided differences in cake volume and internal cake characteristics. It is evident that a coarse flour is less evenly distributed in a batter emulsion, which has a tendency to weaken rather than strengthen the gel structure. When high-ratio formulas are used, it is very important that the flour be finely milled and of the proper analysis and granulation to give the best results.

A survey was conducted primarily to check the granulation of the various flour streams which are selected for definite cake flour types,

¹ There is danger that these statements and quotations may lead to confusion of "starch granule size" and "granulation." These terms are in no way synonymous. Starch granule size refers to the size of the individual starch granule which is not determined by grinding or bolting. Granulation refers to the size of the flour particles and is determined by milling and bolting procedures. A flour granule contains a large number of starch granules.

and to note any improvement by using finer silks as affecting the flour quality. The Ro-Tap testing sieve shaker was used in the experiment, and the percent of stocks remaining on the sieves after each 10, 20, 30, and 45 minute operation was recorded. Carmichael cloth cleaners were used on each sieve in order to facilitate the bolting operation. The moisture present in the flour streams tested seemed to be a critical factor, and in order to eliminate this as much as possible all the samples were dried to approximately 11%. It was found necessary to continue the bolting operation on many of the lower grade streams for 60 minutes, and then some difficulty was experienced in obtaining good replications. This was due in part to the soft gummy and flattened condition of the clear and low grade stocks. In place of the standard silk bolting cloth which is normally used in the flow of a mill, we substituted metal sieves which were permanently fastened to eight-inch metal frames. The average dimension of the aperture opening and mesh of the metal sieves are given in Table I together with the equivalent in silk bolting cloth.

TABLE I
SIEVE DIMENSIONS

Mesh and opening—wire cloth	Equivalent openings to silk
165 mesh Dur-Loy bolting cloth, 0.0042" opening	12XX or 13XX standard silk
230 mesh Dur-Loy bolting cloth, 0.0029" opening	21XX standard silk
250 mesh Phosphor Bronze cloth, 0.0024" opening	25XX standard silk

Courtesy W. S. Tyler Company.

The results shown in Table II indicate the variation found in a few of the mill streams listed. We have excluded from this series many of the higher-ash streams which are normally classified as clear and low

TABLE II
GRANULATION OF FLOUR STREAMS OBTAINED ON RO-TAP SIEVE SHAKER IN 45-MINUTE OPERATION

Mill stream	Ash (%)	Granulation (%) (throughs of 250 mesh)
1st middlings	0.275	97.0
2nd middlings	0.280	96.0
3rd middlings	0.295	93.0
4th middlings	0.325	89.0
5th middlings	0.350	84.0
6th middlings	0.385	80.0
7th middlings	0.505	72.0
Sizings	0.305	97.0
1st break flour	0.400	98.0
2nd break flour	0.360	97.0
3rd break flour	0.355	97.0
4th break flour	0.445	83.0
5th break flour	0.620	70.0

grades. The mill streams tested seemed important in that the lower-ash streams which comprised approximately 40% to 50% of the total flour gave a very satisfactory granulation. The ash test is given only as an indication of the refinement of the mill streams, as this factor cannot be correlated with the granulation. This is clearly shown when we compare the break and middling flours of nearly the same ash content. The break flours, which are usually low in viscosity, do reflect a satisfactory granulation, although they are usually somewhat higher in ash depending upon the flow of the mill. The low-ash middling flours are higher in viscosity, and should receive due consideration if any changes are contemplated in the flow of the mill in order to obtain a finer flour.

The results of Table II are interesting in comparing flours of different extraction, in that various streams which may be included will reflect a definite trend in granulation. For this reason we feel that a short patent cake flour of 40% to 50% extraction is finer than flours which have been milled to a 95% extraction. This may partially explain the preference of bakers who demand short-patent cake flours over the longer extractions. It is not uncommon during the year to obtain some wheat which is hard and flinty in character. Wheats of this type when milled will reflect a notable change in the granulation of the flour. It is for this reason that great care should be exercised in the purchase of wheat so that it will give the desired characteristics when milled into flour. Abnormal ripening conditions of the wheat during the growing season, variety, and a number of other factors may change materially the granulation from one crop to the next. It is important therefore to check the flours very carefully, and to maintain a definite standard on all grades.

TABLE III

ANALYSIS OF FLOUR STOCKS BOLTED FROM A SHORT-PATENT CAKE FLOUR IN 45-MINUTE OPERATION OF RO-TAP SIFTER

Analysis	Short-patent flour (standard)	Stock remaining on 250 mesh	Stock through 250 mesh
Ash, % ¹	0.345	0.355	0.342
Protein, % ¹	7.90	9.50	7.50
Viscosity, No. 1. ²	23	76	20
Viscosity, No. 2. ²	31	85	25
pH	5.00	5.10	5.00

¹ Ash and protein reported on 15% moisture basis.

² Viscosity reported by No. 1 (no-time method) and No. 2 (1-hour digestion method) in degrees MacMichael.

The data shown in Table III indicate a difference between the analysis of the stock remaining on a 250-mesh sieve and the very fine flour which was bolted through the same mesh. This difference in

granulation may be due to the fact that in a wheat mixture a very small percentage of hard vitreous kernels present are not reduced properly in the milling process. It is for this reason that careful selection of the wheat should be made before undesirable grades have been purchased.

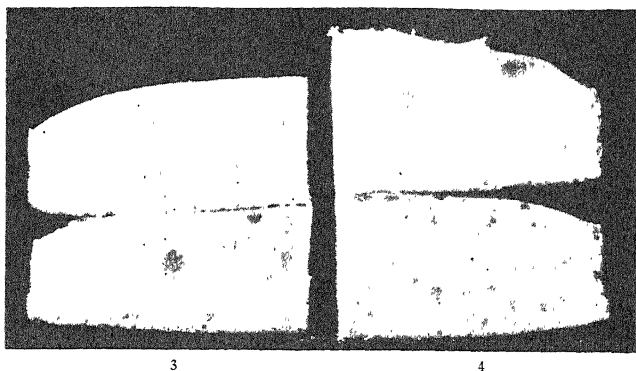


Fig 1 Shows the results obtained by the cake test of the stock remaining on a 250-mesh (cake No. 3) as compared with the fine stock bolted through a 250-mesh (cake No. 4).

Cake tests shown in Figure 1 indicate the relative importance of granulation in cake flours. The recommended tentative basic-cake formula of A. A. C. C. was used.

Summary and Conclusions

The granulation test has been found useful in standardizing the degree of fineness to which flours are milled, and is therefore recommended as a laboratory routine test. Granulation may be considered as one of the factors which seem to influence the baking characteristics of a cake flour, although not essentially the most important one.

Short-patent cake flours seem better suited for high-ratio formulas, primarily because of the better quality of the streams selected and the finer granulation which is present in these streams.

The character of the flour gluten and percent of flour extraction, protein, viscosity, and pH are all obviously important, but these alone will not insure optimum baking results unless the flour has been milled to a predetermined degree of fineness.

The granulation of a flour seems more dependent upon the proper balance of the flour streams in the bolters and purifiers, than upon the fine bolting silks which are used in the flow of the mill.

The comparative effects of differences in granulation of the mill streams selected are in accordance with the observations of those who have previously contributed to this subject.

Literature Cited

- Alexander, G. L.
1939 Soft-wheat testing problems. *Cereal Chem.* 16: 197-208.
- Alsberg, C. L., and Griffing, E. P.
1925 Effect of fine grinding upon flour. *Cereal Chem.* 2: 325-344.
- Hastings, P. O.
1938 Starch as a factor in cake flour quality. *Association of Operative Millers, Bulletin* 31, p. 922.
- Jones, C. R.
1940 The production of mechanically damaged starch in milling as a governing factor in the diastatic activity of flour. *Cereal Chem.* 17: 133-169.
- Sandstedt, R. M., Jolitz, C. E., and Blish, M. J.
1939 Starch in relation to some baking properties of flour. *Cereal Chem.* 16: 780-792.
- Woodruff, S., and Nicoli, L.
1931 Starch gels. *Cereal Chem.* 8: 243-251.

REPORT OF THE 1940-41 COMMITTEE ON TESTING BISCUIT AND CRACKER FLOURS

H. J. LOVING, *Chairman*

The Kroger Food Foundation, Cincinnati, Ohio

(Read at the Annual Meeting, May 1941)

The 1939-40 Committee on Testing Biscuit and Cracker Flours recommended that (1) further work on cracker flours be suspended until the statistical study could be prepared covering the five years' work done by the committee on that subject, and (2) that work be done on biscuit or cookie type flours in order to bring such tests up to the present status of tests on cracker flours (Simmons, 1941). The statistical study referred to has been prepared and submitted to the committee by one of its members, Mr. Tarnutzer. After appropriate consideration by the committee, it will be placed in final form and submitted for publication.

For the evaluation of cookie flours, the work of this year's committee has taken the form of a collaborative study of the formula and procedure proposed by Alexander (1933).

The flours chosen on which to conduct the cookie-test study were three soft winter wheat flours from the Middle West, the intermediate being a blend of equal parts of the stronger and weaker. Analyses of the three flours employed appear in Table I.

It has been suggested that the performance of cookie flours may be related to particle size. Table II presents data obtained from a granulation study of the three flours. The results seem significant, but in view of strength differences as revealed by protein and viscosity determinations, it is impossible to properly evaluate the influences of particle size distribution.

TABLE I
ANALYSIS OF FLOURS

	Flour No. 1	Flour No. 2	Flour No. 3
Ash (15% moisture basis) %	0.398	0.393	0.402
Protein (15% moisture basis) %	7.81	8.54	9.07
Viscosity, no-time method ¹	27	52	72
Viscosity, 60-min digestion ¹	39	65	91
Viscosity, 2-g protein	49	71	87

¹ Sample weights adjusted to 20 g at 15% moisture.

TABLE II
GRANULATION OF COOKY FLOURS OBTAINED IN 50 MINUTES WITH
RO-TAP SIEVE SHAKER

	Granulation test—%		
	Sample No. 1	Sample No. 2	Sample No. 3
Moisture, %	10.50	10.40	9.80
On 165 mesh, 10 min	X	X	X
On 165 mesh, 20 min	X	X	X
On 230 mesh, 10 min	8	10	13
On 230 mesh, 20 min	2	5	7
On 250 mesh, 10 min	70	71	68
On 250 mesh, 20 min	48	53	48
Through 250 mesh, 30 min	73	66	65
Through 250 mesh, 40 min	86	80	79
Through 250 mesh, 50 min	93	88	84

The flour which was identified to the collaborators as No. 1 was a 94% flour from Michigan white wheat running approximately 9.0% protein. The flour identified to the collaborators as No. 3 was a 95% extraction flour from high-protein Ohio soft red winter wheat. Flour No. 2 was a thorough blend of equal parts of flours No. 1 and No. 3. All three flours were unbleached.

Samples of these three flours, and along with them instructions for applying the cooky formula previously referred to, with slight modifications, were submitted to the committee members. In order to cover sufficient ground to make the study worth while, five possible variables in formula or procedure were chosen, and each of these variables was assigned to either one or two committee members for investigation and possible change in the cooky test.

Dunn (1933) expressed some disappointment in laboratory tests of flours in cookies where "spread" was evaluated. He stated that "In spite of all we could do, there was a lack of uniformity in the spread of these cookies, as well as in the contour and general appearance, which was so great that fine distinctions between flours could not be drawn." It was the idea of the committee that it should be possible

to make adjustments in the formula employed to permit more sensitivity, or sharper differentiation between flours of different strengths and thus minimize such lack of uniformity as familiarity with the test would not prevent. Each collaborator investigated a variable and at the same time conducted bakes on the test as submitted, which gave a common basis for comparison among all operators.

Instructions for conducting the laboratory cookie tests were as follows:

Formula:

Baker's Special granulated sugar	130 g
Hydrogenated fat	64 g (75° F)
Whole egg (fresh)	26 g
Ammonium carbonate	0.5
Soda	2.5
Skim milk suspension	40 ml
Flour	224 g
Salt	2.1 g

28.2 grams of spray-powdered skim milk are mixed into 150 ml of distilled water to make the skim milk suspension.

Procedure: Cream sugar, shortener and soda three minutes, cutting down after each minute (Kitchen Aid Model G, second speed, 128 rpm, or equivalent).

Add eggs slowly during one minute in low speed (low speed on Kitchen Aid, 62 rpm). Scrape down. Mix in second speed for one minute.

Dissolve salt and ammonium carbonate in the skim milk suspension. Add skim milk suspension during one minute in low speed. Scrape down. Mix one minute in second speed. Add whole quantity of flour, mix for two minutes in low speed, cutting down after each half minute.

Place small handfuls of batter at six well spaced points on a cookie sheet so that the cookies when cut will be about two inches apart. Make sure that each handful of batter is coherent and not composed of different scraps pressed together, as this latter practice tends to produce imperfect cookies. Lay wooden strips 7 mm in thickness along each side of the cookie sheet and roll the batter out with rolling pin to this height. Cut a cookie in the center of each piece with the cutter provided (lower part of ointment tin) which has a diameter about 60 mm. Remove scrap and discard, leaving cookies in place ready to be baked.

Bake cookies at 400°F for 10 minutes. On removal from the oven immediately lift cookies from pan to cooling rack or absorbent paper.

After 30 minutes inspect the cookies and measure thickness and width. Thickness can be best measured by piling six atop one another and averaging the height. Average diameter should be obtained by making two measurements of diameter on each cookie.

The spread factor W/T should be computed, W being average diameter and T the average thickness. The greater this factor, the more spread possessed by the cookies.

A score form was provided each collaborator, requiring qualitative evaluation of symmetry, regularity of edge, top color, top grain, bottom appearance, and grain and texture.

Discussion of Results

C. C. Armuth investigated sugar levels over a range of minus 10% of the amount given in the formula to plus 10%. Distinctly more spread was seen at the 10% higher sugar level, and distinctly less spread at the lower sugar level, as compared with the standard formula.

The mixing stage after addition of flour was given in the instructions as two minutes in low speed on a Model G Kitchen Aid (about 62 rpm). The variable investigated by C. E. Bode constituted mixing times of one to three minutes, or a variation of plus or minus 50%. Widest differentiation between the three flours was exhibited at $1\frac{1}{2}$ minutes of mixing time. Preference was expressed by this collaborator for the cooky made at $2\frac{1}{2}$ minutes of mixing time. He also favored the use of second speed in this operation as yielding cookies easily differentiated as well as being expedient in preparation. The actual amount of spread obtained did not vary greatly with the degree of mixing employed.

Variation in liquid content of the formula from minus 10% to plus 10% of the quantity called for was investigated by Miss Brown. Correct differentiation between the flours was made in practically every series of bakes, although the influence of changing liquid level was not as pronounced in spread as was expected by this collaborator.

W. H. Hanson varied mixing time at levels of plus 25% and plus 50% of the recommended treatment. This collaborator stated that he could easily identify the weakest flour, but had some difficulty in his differentiation by cooky tests between flours No. 2 and No. 3, and also that the cooky spread seems very definitely associated with wheat variety to a large extent. Work with the granulation test may reveal that it is a factor in milling the same variety of wheats, as reflected by the cooky test. As was noted in the case of the other collaborator investigating the same variable, the changes in mixing time did not greatly affect the absolute spread experienced.

Liquid variations as well as oven conditions were investigated by T. E. Hollingshead. The liquid levels utilized were from minus 10% to plus 20% of the quantities originally specified. Other conditions being equal, this collaborator found that the higher the liquid level, the more the spread, and conversely. As was noted by at least one other operator, Hollingshead noted that much of the spring taken on by the cookies in the oven was lost when they were removed and allowed to cool. This frequently left a lumpy or irregular top which contributed to variations in measuring average thickness. Bakes were made wherein top heat was used for the last five to seven minutes of oven treatment, which successfully baked the cooky tops and caused them to retain the complete spring gained in the oven, even after cooling. It was stated by this collaborator that the addition of 15% additional water to the original formula gave the maximum spread consistent with good texture.

The investigation of the influence on spread and pH contributed by variations in soda was conducted by H. M. Simmons. As would

be expected, increase of soda increased alkalinity and decreased soda had the opposite effect. Changes in soda level made some modifications of spread (width and thickness) and crispness. Characteristics such as top color, bottom appearance, or shape seemed to be unmodified. In general, spread was greater with increase of soda and less with decreases of soda. It was the recommendation of this collaborator that the soda in the formula be maintained at the stipulated level.

Shortener quantities were varied from plus 20% to minus 30% of the standard formula by C. A. Tarnutzer. In addition to the regular score factors suggested by the committee chairman, this collaborator also included some others. It was concluded that the count per pound would not correlate with differences in flour strength. Tarnutzer was quickly able to point out flour No. 1 as a medium to weak cookie flour. Proper designation of the strength ranking of flours No. 2 and No. 3 was also made, but some confusion was apparent in the light of the cookie test results. It was the conclusion of this collaborator that as a single test for flour strength, the cookie test with which the committee was working should be modified to the leanest level here investigated, namely a reduction in shortener content of 30%.

Sugar variation from plus 10% to minus 15% of the recommended formula was investigated by H. O. Triebold. It was his conclusion that the standard formula minus 10% or minus 5% of sugar was the most useful in differentiating the three flours.

Summary and Conclusions

Of the ten series completed with the recommended formula and procedure, six ranked the three flours in order of their strength according to protein and viscosity determinations (Table III). Twenty-five

TABLE III
SUMMARY OF SPREAD FACTORS ON TEN MIXES COMPLETED WITHOUT
VARIATION OF STANDARD CONDITIONS

Collaborator	Flour No. 1			Flour No. 2			Flour No. 3		
	Average W (mm)	Average T (mm)	Average W/T	Average W (mm)	Average T (mm)	Average W/T	Average W (mm)	Average T (mm)	Average W/T
Armuth	82.0	11.17	7.34	84.0	11.33	7.41	84.0	10.5	8.00
Armuth ¹	84.0	10.33	8.13	82.0	12.83	6.41	81.8	12.0	6.81
Bode	84.4	11.8	7.15	83.3	12.33	6.75	81.0	13.17	6.15
Brown	77.3	12.0	6.4	77.2	12.75	6.1	72.2	14.2	5.3
Hanson	88.7	12.0	7.39	85.4	12.67	6.74	83.4	13.33	6.26
Hollingshead	83.1	9.3	8.94	79.4	9.7	8.19	80.1	10.5	7.73
Simmons	78.0	10.2	7.67	77.5	11.7	6.65	77.0	11.3	6.87
Simmons	80.3	11.7	6.88	79.6	13.0	6.11	78.8	13.0	6.06
Tarnutzer	81.9	11.94	6.86	80.3	13.61	5.90	80.0	14.20	5.63
Triebold	83.1	12.1	6.9	98.1	11.2	8.8	82.5	11.3	7.3

¹ Different oven employed.

series of the total of forty-four (56.9%) properly ranked the three flours. Most of the exceptions where the proper order was not apparent from the cooky test arose in a confusion of flours No. 2 and No. 3. This will be shown by the fact that 11 of the 44 bakes completed would rank the flours in ascending order of strength as follows: No. 1, No. 3, No. 2.

While admittedly this differentiation between flours varying distinctly in strength is far from being as accurate as certain of our other tests, it is felt that some promise is shown, particularly when we take into account the fact that most of the collaborators were entirely unfamiliar with the test at the beginning of the year's work. Almost without exception each collaborator expressed belief in the value of the test as one suitable for the evaluation of the cooky-making properties of soft wheat flours.

Taking the comments of the various collaborators regarding the variable investigated in each case, we can conclude that modification of the original formula to give greater sensitivity and sharper differentiation between flours of varying strengths could be expected by employing a higher moisture level and by reducing the quantity of shortener and perhaps sugar. It seems to be indicated that little or nothing would be gained by modifying the soda percentage and pH, and indications are that mixing time is about right if these same mixer speeds are employed. However, some interest was expressed in reducing the amount of time for completing the mix by substituting lower times with higher speeds. Some success along this line was reported by Bode and Simmons.

In more than one case objection was expressed to the fact that cookies from the oven immediately developed uneven top shrinkage, leaving a lumpy or irregular "profile," as it was expressed in the cooky inspection report submitted by one of the collaborators. The use of top heat in the oven for the final minutes of baking properly surmounted the criticism and left the entire oven spring in the cooled cookies to be evaluated in the spread factor, which was obtained by dividing the average diameter by the average thickness of six cookies. One collaborator also recommended that the exact quantities of milk and water always be weighed for each batch, inasmuch as proper volume measurement was interfered with by the foaming properties of the suspension.

Recommendations

The work of the committee for this year provided too small a body of data to permit entirely valid conclusions as to the value, reliability, and sensitivity of the tests investigated.

The committee recommends at least one more year of purely laboratory work along these same lines, modified in the direction of higher liquid and leaner formulas generally. It is obvious that greater familiarity will be necessary with this test before it will be a reliable instrument for evaluating flour quality for functions where cookies of the type exhibiting spread are desired.

Acknowledgment

The chairman of this committee expresses here his appreciation of the excellent cooperation afforded by the eight active members of the committee. Expression of appreciation is also made of the work done by E. G. Kornreich of the Kroger Food Foundation and William T. Hanks of the Technical Institute.

Literature Cited

Alexander, G. L.

1933 The results of bleaching Michigan soft winter wheat cake flours by the Brabender electric bleaching apparatus. *Cereal Chem.* 10: 623-626.

Dunn, J. A.

1933 Testing biscuit and cracker flour. *Cereal Chem.* 10: 628-631.

Simmons, H. M.

1941 Report of the 1939-40 committee on testing biscuit and cracker flour. *Cereal Chem.* 18: 252-257.

REPORT OF THE 1940-41 COMMITTEE ON TESTING SELF-RISING AND PHOSPHATED FLOURS

ELMER MODEER, *Chairman*

The St. Joseph Testing Laboratories, Inc., St. Joseph, Missouri

(Read at the Annual Meeting, May 1941)

As the major problem for the 1940-41 year, the committee decided to investigate the practicability of adapting the present biscuit-baking test, by modifying either the formula or the procedure, to enable anhydrous calcium acid phosphate, as well as the hydrated form, to be used. Secondly, the committee sought further data regarding the present baking test and scoring plan as a means of intra-laboratory characterization of self-rising and phosphated flours.

In the past several years, the use of anhydrous calcium acid phosphate as a leavening agent has become commonplace, so that if possible the ability to interchange the two phosphate forms would be a great convenience.

Accordingly, a series of self-rising flours was prepared, one containing regular hydrated phosphate, in the usual official formula, the others containing varied amounts of anhydrous phosphate, with soda, of course, in proportion. A single-base flour was used throughout, a pure soft Missouri standard patent being selected as a representative type. The analysis of the flour was 9.2% protein, 0.39% ash, viscosity

88° McM, and pH 5.68. It was intended that the flour be bleached with chlorine and benzoyl peroxide, but it was not discovered until after the project was started that the chlorine had been omitted, which doubtless accounts for the relatively low score by the regular method.

The regular hydrated formula sample, designated No. 1, was prepared according to the formula of Walter (1936), namely 1.87% calcium acid phosphate hydrate, 1.5% sodium bicarbonate, 2% sodium chloride based on flour weight.

Samples designated No. 2, No. 3, and No. 4 contained, respectively, 1.5%, 1.3%, and 1.1% anhydrous calcium acid phosphate, and 1.25%, 1.08%, and 0.91% soda, the first being the commercially recommended percentage. All three contained 2.25% salt.

The composite results of the six reporting collaborators are shown in Table I.

TABLE I
COMPOSITE BISCUIT SCORE

	Standard	Samples			
		1	2	3	4
Grain	10	8.0	8.8	8.7	8.3
Tenderness	10	8.0	9.7	9.5	8.5
Flavor	20	18.0	18.1	18.5	18.0
Crumb color	20	16.3	17.9	17.7	17.1
Volume	40	42.6	48.0	45.9	44.2
Total score	100	92.9	102.5	99.3	96.1
pH	—	7.25	7.31	7.23	7.14
Oven loss, %	—	13.2	13.3	12.9	12.2
Specific volume	—	2.13	2.40	2.33	2.21

The committee is in general agreement that the No. 2, No. 3, and No. 4 bakes are not in agreement with No. 1 in any important respect, and actual differences in characteristics are greater than can be revealed in tabulated columns of figures. The handling characteristics of the doughs were different; in general, anhydrous formula doughs are more critical in time-relationships during mixing, rolling, and cutting, contributing to a greater experimental variation. This is evidenced by a range in specific volume among the collaborators of 0.45 for the regular formula, and 0.48, 0.62, and 0.71 for No. 2, No. 3, and No. 4.

In view of the widely different behavior of the two phosphates, it was not deemed necessary to investigate formally a modification of procedure as well as of formula. Because the specific gravity of anhydrous phosphate doughs is greater than hydrated doughs, the possibility of modifying dough thickness by altering roller stick height to bring results into closer agreement was at first considered. Gookins and

Barackman¹ have both studied the problem. The dissimilar leavening action of the two phosphates, as in the case of formula modification, makes it impossible for equal results to be achieved by a simple modification of this nature.

Variation in scores and analytical data, in general, was normal. With the exception of one collaborator, pH determinations were close, and total biscuit scores agreed to an extent comparable to past years. The factor which showed the widest spread, and which in turn doubtless affected biscuit characteristics, was oven loss. The collaborative data for oven loss are shown in Table II.

TABLE II
OVEN LOSS

Collaborator	Samples				Average
	1	2	3	4	
	%	%	%	%	%
A	9.4	8.7	7.8	7.9	8.4
B	15.1	15.0	14.9	15.7	15.2
C	7.4	9.3	7.1	8.5	8.1
D	11.9	11.1	10.3	9.1	10.6
E	16.4	16.6	15.4	16.7	16.3
F	19.0	19.3	17.6	19.5	18.9
Average	13.2	13.3	12.9	12.2	—

This wide variation in oven loss is symptomatic of oven differences which unquestionably affect biscuit character in all respects. It was shown by the 1939-40 committee (Gookins, 1941) that baking to a uniform oven loss did not improve biscuit volume agreement among laboratories. It appears logical, however, that while volume and loss are not directly related, they are related to the same thing, oven environment. Baking to a constant oven loss would not correct all oven differences any more than, for example, baking yeast bread to a common crust color would compensate for all differences in bread ovens.

Conclusions

From a study of hydrated and anhydrous calcium acid phosphate in self-rising test flours the committee finds that the results indicate the characteristics of the two phosphates are much too different for interchangeable use, by modifying either formula or method, so that it is recommended that for intra-laboratory checking for intrinsic self-rising properties of flour, hydrated phosphate and the present recommended method be employed. Secondly, the results of the committee's collaborative baking and scoring indicate that oven differences,

¹ Private communications.

especially as shown by oven-loss percentages, remain as one of the chief causes of variation among laboratories, a fact which may profitably engage the attention of future committees.

Acknowledgments

Members of the current committee were Lowell Armstrong, R. A. Barackman, O. E. Gookins, Jr., Art King, Elizabeth McKim, F. R. Schwann, and Elmer Modeer. Special acknowledgment is due Mr. Gookins of the committee and Kenneth Spiers of the St. Joseph Testing Laboratories for able advice and assistance.

Literature Cited

- Gookins, O. E.
1941 Report of the 1939-40 committee on methods of testing self-rising and phosphated flours. *Cereal Chem.* 18: 258-261.
- Walter, H. G.
1936 Report of the 1934-35 committee on methods of testing self-rising flours. *Cereal Chem.* 13: 722-723.

THE APPLICATION OF VARIOUS BAKING TEST METHODS TO THE EVALUATION OF SOFT WHEAT

J. A. SHELLENBERGER, PAUL W. HODLER, and C. A. NELSON

The Mennel Milling Company, Toledo, Ohio

(Read at the Annual Meeting, May 1941)

Baking tests for the appraisal of soft wheat flours are numerous. *Cereal Laboratory Methods* (1935) of the A. A. C. C. outlines baking procedures for bread, cakes, biscuits, and pie crust. The cookie test (Alexander, 1933) should also be included in this list. Theoretically, flour quality should be evaluated by testing the flour for each specific baking purpose. This idealistic concept has many practical limitations. For example, many cereal laboratories are required to estimate the quality of a large number of wheats in a limited period of time, and this precludes an exhaustive study of each flour sample. It is known that each of the baking tests has some merit for evaluating flour, but it is questionable which one, if any, of the tests is capable of providing an adequate index of soft wheat quality. There is need for information which will relate and compare the various baking procedures. The ideal test would differentiate adequately between samples and also would indicate the suitability of the flour for specific commercial use.

The present investigation was undertaken for the purpose of making a comparison between the various baking tests on a series of flours milled from soft wheats. The wheat samples studied were known to differ genetically and to have been grown in different regions.

One of the major considerations in baking tests on experimentally milled wheat samples is to bring the flour to such a condition that its full baking potentialities will be evident. Failure to do this may lead to erroneous conclusions. In this study we have attempted in each case to treat the experimentally milled flour in a manner which would make it comparable with commercial flours used for similar baking purposes. A constant effort was made to test each flour under favorable conditions regarding age, moisture, bleach, and pH for each particular baking procedure.

Description of Material

Twelve samples of soft wheat were obtained from Indiana, Michigan, and Ohio. Ten of these samples were of known variety and two were mixtures from commercial elevators. Two of the samples were soft white wheat and the other ten were soft red winter. The samples are not designated according to variety, since too few tests were conducted to warrant opinion concerning the several varieties included. Thorough baking tests of soft wheat varieties have already been reported by Fifield, Bode, and Bayles (1936) and Bayfield and Shiple (1937).

The wheat samples all had fairly high test weights, the usual protein range, and did not vary greatly in ash content. The samples were normal in all respects. This information is recorded in Table I.

TABLE I
DESCRIPTION OF THE WHEAT AND FLOUR SAMPLES¹

Reference no.	Wheat			Flour				
	Class	Test weight	Protein (N X 5.7)	Protein (N X 5.7)	Ash	Viscosity	Diastatic activity	Gassing power
		lbs	%	%	%	^o MacM	mg	mm
A	SRW	61.2	9.3	8.02	0.35	76	81	166
B	SRW	62.2	9.7	8.13	0.38	91	171	255
C	SRW	62.0	9.9	8.23	0.37	62	122	214
D	SRW	60.0	9.1	7.68	0.32	64	91	185
E	White	60.1	9.0	7.29	0.34	35	97	185
F	SRW	61.0	10.3	8.23	0.32	55	106	214
G	SRW	61.8	10.3	8.34	0.33	96	109	194
H	SRW	61.6	9.5	7.74	0.38	70	90	186
I	SRW	60.5	11.2	9.03	0.34	80	94	167
J	White	60.0	10.5	8.26	0.34	32	88	183
K	SRW	60.4	9.8	7.93	0.36	69	98	195
L	SRW	59.8	9.4	7.73	0.38	58	88	148

¹ Results reported on 15% moisture basis.

The samples were milled on a Buhler mill (Ziegler, 1938) and a fairly short patent flour removed. The flow of the mill was modified

from the original by the addition of scalping screens on the first and second reduction systems. These scalpers played an important part in producing low-ash flour without the necessity of taking too short a patent for practical purposes. Flour yields of approximately 60% were obtained. In Table I are recorded the protein, ash, viscosity, diastatic activity, and gassing power of these flours.

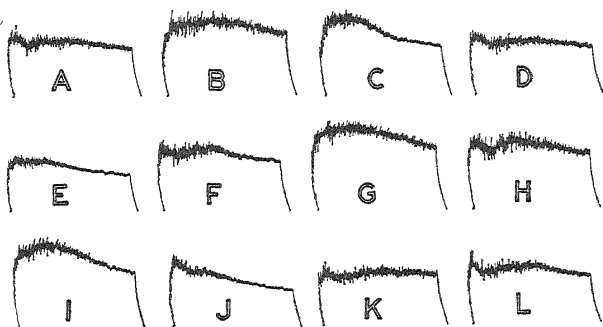


Fig 1 Micro recording dough mixer curves of soft wheat

In Figure 1 are shown the Swanson-Working micro recording dough-mixer curves for the various samples. Soft-wheat curve characteristics have not been stressed to the same extent as have the characteristics of hard-wheat curves. Limitations of the mixing and damping mechanisms on the original recording dough mixers made these machines much less flexible for soft-wheat studies than the present type of micro recording dough mixer. It will be noted that the curves approach in magnitude and shape the curves produced from hard wheat flour by Shellenberger (1940). This is the result of using a weaker damping force in order to magnify differences in the curves which would not otherwise be evident. It is readily observed that the physical dough properties of this series of samples vary over a wide range, and it appears that the recording dough mixer might be extremely useful for differentiating between soft wheat varieties. For example, samples *A* and *B* have nearly the same protein content and are both soft red winter wheats, yet their mixing-curve characteristics are decidedly different. Bailey (1940) has recently discussed the value and limitations of physical dough testing methods.

Experimental

Bread-baking tests: The portions of the flour used for the bread-baking test were bleached with a maturing agent and benzoyl peroxide. The diastatic activity of each sample was adjusted by the addition of

malted wheat flour to approximately 135 mg of maltose as determined by the Blish and Sandstedt (1933) method. As indicated in Table I, the flours originally varied from 81 to 171 mg maltose and the corresponding gassing power varied from 148 to 255 mm pressure. Sherwood and Bailey (1926) called attention to the importance of diastatic activity control and its relation to the baking test. More recently, a number of investigations (Landis and Frey, 1933, and Landis, Frey, and McHugh, 1935) have stressed the desirability of eliminating diastatic activity as a variable factor in test baking.

In accordance with the foregoing suggestions, all the flours used in these baking tests, with the exception of sample *B*, were adjusted to approximately the same diastatic activity. Sample *B* had an initial value of 171 mg of maltose, which is unusually high for soft-wheat flours. It was not deemed advisable to adjust all the samples to this level; therefore, this one flour is out of harmony with the others in respect to diastatic activity and gassing power.

The bread-baking procedure followed in most respects the suggestions made by Bayfield and Shiple (1937) for adaptation of the A. A. C. C. basic bread-baking test for soft wheat evaluation. Four percent sugar, 2% shortening, and 0.50 mg of bromate were used, and the doughs were mixed for two minutes in the Swanson-working mixer. It has been shown by Shellenberger (1938) that more than a one-minute mixing period is required to incorporate completely the dough ingredients. A National Manufacturing Company sheeting roll was used to punch the doughs and also to sheet the doughs before panning.

TABLE II
COMPARISON OF BAKING RESULTS

Reference no.	Absorption ¹	Bread		Cake		Biscuit		Cooky			Pie
		Loaf volume	Bread score	Cake volume	Cake score	Biscuit sp vol	Biscuit score	Average diameter, W	Average thickness, T	Ratio of W/T	Pie crust score
A	51.7	565	91	705	96.0	36.1	78.1	78.6	14.0	5.62	68.0
B	52.5	525	87	695	95.0	33.8	74.8	80.0	14.0	5.71	74.5
C	51.4	505	87	732	95.9	36.6	79.1	74.6	15.0	4.97	68.0
D	51.3	535	89	675	97.6	35.9	75.9	79.4	13.4	5.93	74.0
E	50.0	500	83	635	96.5	37.7	80.2	82.0	12.8	6.41	77.5
F	50.1	565	91	738	96.9	36.3	77.3	81.4	13.8	5.89	75.0
G	52.3	530	88	717	96.8	35.2	77.0	78.8	14.8	5.33	74.0
H	51.1	545	88	730	96.0	35.9	76.7	78.6	15.0	5.24	77.0
I	51.2	595	90	702	95.8	35.7	77.5	79.2	15.2	5.21	71.5
J	49.4	445	78	685	96.3	35.0	77.1	81.6	13.8	5.92	78.5
K	51.5	540	88	745	96.8	34.3	75.3	81.0	15.0	5.40	70.5
L	51.4	540	88	680	96.5	35.9	76.7	79.2	14.0	5.66	75.0

¹ On 15% moisture basis.

Preliminary bakes were used to determine the optimum absorption for each flour sample.

In Table II are recorded the baking results obtained from the twelve flours, and pictures of the bread are shown in Figure 2. The bread

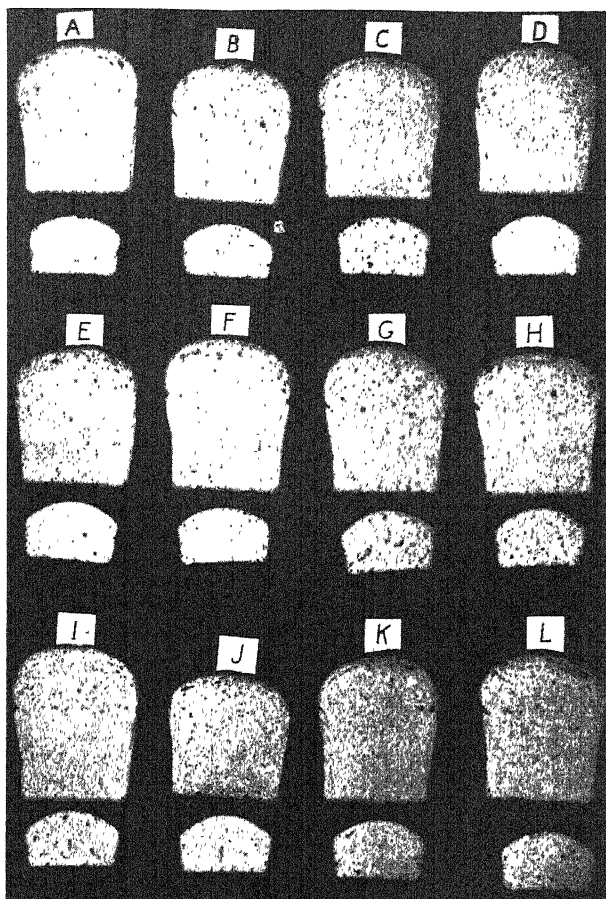


Fig. 2. Photograph of bread and cake made from the different wheat samples.

score was acquired by assigning numerical values to the important internal and external loaf characteristics. The items considered on the basis of 100 for a perfect score were volume, shape, bloom, break, color, grain, and texture.

The doughs all had typical soft-wheat mellowness and possessed, to only a slight degree, those dough properties which are characteristics for good bread production. The two white wheat samples had the weaker and poorer dough quality from the standpoint of bread production. These two wheats also had the least absorption and produced the smallest loaf volumes of the entire series. There were sufficient differences between the external and internal features of the different loaves in most instances to justify a distinguishing score. It is, however, definitely more difficult to differentiate between different soft-wheat flours by the bread-baking test than between unlike hard wheat flours.

Biscuit tests: The biscuit tests were conducted on a portion of the same bleached flour that was used for the bread-baking test. The baking technique followed was the same as that outlined by the Cincinnati Section, A. A. C. C. Research Committee (Schwain 1940). The formula was as follows: 125 g flour (15% moisture basis), 1.88 g soda, 2.34 g mono-calcium phosphate, 2.19 g salt, and 20.0 g shortening. The experience of the Cincinnati Section Research Committee indicated that the foregoing shortening level is near the optimum for soft-wheat-flour biscuit testing purposes. Preliminary bakes were used to determine the optimum absorption. The biscuits were scored by evaluating the various items enumerated by McKim and Moss (1939) and the numerical score assigned according to the A. A. C. C. score card as follows: grain 10, tenderness 10, flavor 20, crumb color 20, volume 40.

The volume and score of the biscuits baked from the various samples are recorded in Table II.

Cake tests: It is generally agreed that for the best cake results a flour should possess the following attributes: low moisture content, fine granulation, and a pH between 5.2 and 5.4. Before the cake-baking tests were started, the samples of flour were made to conform to the previously mentioned conditions. Portions of the original unbleached flour which had been milled on a Buhler mill were further reduced by use of the mill described by Libby and Shellenberger (1938) until 75% bolted through a 16XX silk. The overs and throughs were combined and rebolted through a 9XX to insure thorough re-mixing. No portion of each original sample was discarded.

The flour thus obtained was bleached with benzoyl peroxide and brought to a pH of approximately 5.2 by the use of chlorine. The actual pH range was found to vary from 5.19 to 5.34. The flour samples were all dried to the uniform moisture level of 11.8%.

The A. A. C. C. cake flour baking test was used and the cakes were scored according to the method suggested in *Cereal Laboratory Methods*

(1935). The volumes and scores are recorded in Table II, and in Figure 2 the cakes are compared with the bread baked from the same flour.

Cooky tests: Unbleached flour was used in the cooky test. The sugar cooky formula adopted was that recommended by Alexander (1933) as modified by Fifield, Bode, and Bayles (1936). It was necessary to reduce the absorption slightly in order to obtain a workable dough from these very soft wheat flours.

The cookies made from the various flours were remarkably similar, and it was very difficult to find distinguishing characteristics for grading purposes. Attempts to assign a numerical score to items such as symmetry, color, top grain, appearance, and crumb grain were abandoned. It was fairly obvious, however, that the two white wheat flours produced excellent cookies. In Table II the average thickness (T) and the average diameter (W) of the cookies are recorded and factor W/T is computed. The ratio of diameter to thickness is an index to the spread of cooky doughs, and consequently is an important criterion of quality. The cookies baked from white wheat flour had good spread.

Pie test: Unbleached flour was used in the pie-crust test. Bleached flour frequently produces pale pie crusts which are considered less desirable than when the color is slightly brownish. For this study the pie crust formula and method were those recommended by Kress (1932, 1935), except that these flours required less absorption than those investigated by Kress.

The pies were graded 24 hours after removal from the oven, and numerical values were assigned to the following qualities: dryness, color, tenderness, flakiness, and form. The relative score of the various pies is indicated in Table II.

Discussion of Results

A study of Table II indicates the difficulties and complications encountered in an attempt to evaluate soft wheat by test baking. There appears to be no very definite relationship between the various baking procedures. For example, the lack of conformity between the volumes of the bread and cake and the scores is evident in Figure 2 and Table II. One noticeable feature is that the two white wheat flours, namely *E* and *J*, are deficient in volume according to the bread-baking test, but are satisfactory in this respect when used in cakes. There is frequently an inverse relationship between bread and cake volumes obtained from the same flour, but there are many exceptions to this rule. Considered entirely from the standpoint of differentiating between soft-wheat flours of nearly the same quality, the bread-baking

test is preferable to the cake-baking test. The distinguishing characteristics of bread are generally more pronounced than the corresponding qualities of cake. Another item of no small consequence is the greater ease with which the bread-baking test can be carried out in comparison with the ease in conducting the cake-baking test.

There are a number of ways in which the bread-baking test can be used advantageously to predict the cake-baking quality of flour. When a cake flour has been evaluated by actual cake production, it is usually possible to establish by comparative bread-baking tests a series of dough and loaf characteristics which can be regarded as typical for that type of cake flour. The standards thus created can be used with convenience and reliability.

The fact that all samples except sample *B* gave fairly consistent results by the cake-baking test might justly raise a question concerning the value of the cake-baking procedure used. A baking test is of greatly diminished value if it will not differentiate between flour samples which have been milled from wheat known to be of different genetic make-up and grown under diverse climatic and soil conditions. It is not sufficient that the baking test detect poor results when bread flours or other unadapted flours are used in cakes. More convenient means are available for this purpose. A cake-flour test, to be of value, must be able to differentiate between flours which are all reasonably adapted to that purpose. Results of this series of tests, as well as other observations, lead to the conclusion that our present cake-baking test will not satisfactorily meet the foregoing condition. It may be that because of the ratio of other ingredients to flour in the cake-baking test, it can never be successfully adapted for the purpose of differentiating between flours of approximately the same quality.

The biscuit-baking test appears to classify flours entirely independently of their suitability for either bread or cake. The biscuit test was not especially critical and did not differentiate adequately between flours of the same general character.

The pie-crust baking test can also be included in the same category. The pie crusts from the twelve samples were very similar in most respects, and consequently the scoring was difficult and subject to considerable differences of opinion. One point worthy of special emphasis is the parallelism between the pie-crust score and the spread of cooky doughs as indicated by the factor W/T previously discussed. It is generally agreed that the greater the quotient obtained by dividing the average diameter by the average thickness of the same cookies, the better the cooky. Indications are that the cooky test provides a good indication of the desirability of a flour for pie crust and other pastries where spread and shortness are concerned. The

disadvantages of both the cooky and pie tests are the time-consuming operations required to perform the tests. It appears, however, that neither the bread, cake, nor biscuit tests can be used satisfactorily as a basis for predicting the suitability of a flour for doughs that require spread and shortness.

The present study also appears to indicate that the viscosity test is not closely associated with the various baking tests. Reference to Table I shows that the viscosity of the flour samples varied from 96° to 32° MacMichael. This viscosity range should correspond to a gradation in baking value, but as indicated by the scores recorded in Table II for the various baking tests, no very definite relationship exists.

Bayfield (1934, 1935) and Bayfield and Shiple (1937) made extensive studies of the methods of evaluating experimentally milled soft-wheat flours, and the viscosity test received considerable attention. As a measure of protein quality, it proved to be a promising but not entirely conclusive indication of flour strength. The viscosity test is extremely useful for differentiating between flours of different quality, but it cannot be used successfully without supplementary information to predict baking quality. Admittedly the baking methods used in this investigation have been submitted to a severe test. Possibly there is no justification for expecting baking tests to provide unmistakable evidence of dissimilarity between soft-wheat flours of nearly identical type. Also, in a comparison of methods of evaluating flours, consideration must be given to the fact that the interpretations of a test may perhaps be subject to as much criticism as is the method. Methods for interpreting the results of baking tests on soft wheat should continue to receive adequate consideration.

Cracker Sponge Studies

One of the unfortunate shortcomings of our soft-wheat testing techniques is lack of an informative baking test for cracker sponge flour. Attempts to bake certain types of crackers in the laboratory have never been entirely successful. It is possible to relate information obtained from the bread-baking test to the known suitability of the flour for cracker sponge doughs as determined by commercial use. This approach is of limited value and obviously cannot be very helpful to the plant breeder engaged in developing new wheat varieties that need to be tested for adaptability as cracker sponge flour types.

In an endeavor to follow the physical changes which cracker doughs undergo during extended fermentation, use was made of the micro recording dough mixer. The procedure was as follows: flour-water doughs containing 0.3% yeast and 50% absorption were mixed for 1½ minutes in the Hobart-Swanson mixer. Fifty-two grams of

each of these doughs were placed in the mixing bowl of the micro recording dough mixer and the curves obtained. In Figure 3 the upper curves are the ones obtained at the initial stage.

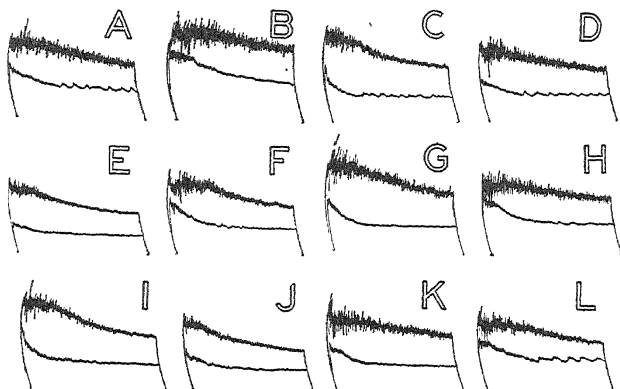


Fig. 3. Micro recording dough mixer curves of cracker sponge doughs before and after 20-hour fermentation period. Upper curves show initial stage.

The remaining portions of the large doughs were placed in a fermentation cabinet at 86°F and allowed to remain undisturbed for 20 hours. Fifty-two grams of the doughs were again weighed into the recording dough-mixer bowl and the curves recorded directly below the corresponding curve which was made when the doughs were first set. This is illustrated in Figure 3. The same damping adjustment was used in each instance.

These curves indicate very strikingly the different degrees of modification that aging brings about in the doughs. This is the result of a number of factors, such as the reorganization of the gluten strands, the effect of accumulated H-ions upon the dough proteins, and the partial proteolysis caused by the enzymes at work on the system. Landis (1935) has recommended the use of dough-mixing studies as an index of proteolytic activity. More recently Swanson (1940) has studied the effect of enzymes on curve characteristics and found, after a period of dough autolysis, that the curves were very similar to those produced by the addition of proteases. It is possible that with additional study the changes occurring in the physical condition of the dough, as indicated by mixing curves, may be correlated with the suitability of the flour for cracker dough production. Sample A, Figure 3, is an example of the curves from a satisfactory cracker sponge flour. The thixotropic condition of the doughs is indicated by

the decreased mobility after a period of quiescence and the subsequent drop in shearing force as the dough becomes a sol. The curves of sample *B*, for example, are noticeably different from those of sample *A*. Sample *E* represents another type of curve where even less strength is evidenced.

Summary and Conclusions

This investigation had as its objective the determination of the relationship between the various baking tests that can be applied to soft wheat. Information was also sought on the degree of differentiation between samples which the various baking tests are able to disclose.

Twelve samples of soft wheat grown under different environmental conditions and differing genetically were obtained. These samples were experimentally milled and several portions of the flour were especially treated to improve their baking response for specific tests. Comparative bread, cake, biscuit, cooky, and pie flour baking tests were conducted and the products evaluated by careful scoring.

Considering the subject entirely from the standpoint of differentiation between flours, the bread-baking test has greater merit than any of the others. This test, however, leaves much to be desired. The limitations of the bread baking test, when applied to soft wheat, have been previously discussed by Bayfield and Shipley (1933) and Bayfield (1934). It is true that the test is not perfectly adapted to soft wheat flour studies, but, as indicated by Shellenberger (1940), it is possible to obtain much valuable information by its use.

Except for the parallelism between the cooky and pie crust tests, there is no apparent relationship between the baking tests. Neither the volumes nor the scores of the bread, cake, and biscuit tests correspond sufficiently to warrant prediction of the baking results of a flour for other than one purpose. Without supplementary evidence or experience applicable to the flour under investigation, it is unwise to attempt the precise evaluation of a flour for purposes other than that indicated by the particular baking test used.

Present baking methods are satisfactory for the broader classification of wheat flours into categories such as bakery, family, and pastry types. The vital need at present is for baking and interpretive methods that can be used with reasonable ease and reliability to predict the relative value of a soft-wheat flour within the broader classification. Our present baking-test methods apparently indicate the potentialities of a flour for only one purpose, thus necessitating the tedious task of conducting separate baking tests for the complete evaluation of the flour.

The micro recording dough mixer is useful for indicating differences in the dough characteristics of soft-wheat flours. There is evidence that this instrument may be useful in helping to characterize cracker sponge flour.

Literature Cited

- Alexander, G. L.
1933 The results of bleaching Michigan soft winter wheat cake flours by the Brabender electric bleaching apparatus. *Cereal Chem.* 10: 623-626.
- American Association of Cereal Chemists
1935 *Cereal laboratory methods* (3rd ed.).
- Bailey, C. H.
1940 Physical tests of flour quality. *Wheat Studies of the Food Research Institute*, Vol. 16, No. 6.
- Bayfield, E. G.
1934 Soft winter wheat studies. II. Evaluating experimentally milled flours with the aid of viscosity, fermentation, and baking tests. *Cereal Chem.* 11: 121-140.
1935 Soft winter wheat studies. III. The effect of some factors influencing viscosity and protein. *Cereal Chem.* 12: 1-16.
- and Shiple, V.
1933 Soft winter wheat studies. I. The suitability of the A. A. C. C. basic baking procedure for the determination of strength. *Cereal Chem.* 10: 140-148.
1937 Soft winter wheat studies. V. Evaluating the quality and the strength of some varieties. *Cereal Chem.* 14: 551-577.
- Blish, M. J., and Sandstedt, R. M.
1933 An improved method for the estimation of flour diastatic value. *Cereal Chem.* 10: 189-202.
- Fifield, C. C., Bode, C. E., and Bayles, B. B.
1936 Chemical, milling, and baking results for wheat varieties grown in the cooperative varietal experiments in the Western Region in 1934. U. S. Department of Agriculture, Bureau of Plant Industry. Mimeographed report.
- Kress, C. B., Chairman
1932 Report of the subcommittee on pie-flour tests. *Cereal Chem.* 9: 411-413.
1935 Report of the 1933-34 pie-flour committee. *Cereal Chem.* 12: 158-160.
- Landis, Q.
1935 An index of proteolytic activity by use of the farinograph. *Cereal Chem.* 12: 25-33.
- and Frey, C. N.
1933 Diastatic supplements for the A. A. C. C. baking test. *Cereal Chem.* 10: 588-592.
- and McHugh, S. A.
1935 Diastatic supplements for the A. A. C. C. baking test. *Cereal Chem.* 12: 302-304.
- Libby, J. J., and Shellenberger, J. A.
1938 Unique experimental mill. *The Northwestern Miller* (Production Number) Vol. 193, No. 7.
- McKim, Elizabeth, and Moss, H. V.
1939 Study of definition and evaluation of various items on score card. *Cereal Chem.* 16: 117-126.
- Schwain, F. R.
1940 Report of the 1939-1940 research committee, Cincinnati section A. A. C. C. Mimeographed report.
- Shellenberger, J. A.
1938 X-ray photographs as a means of determining when a dough is adequately mixed. *Cereal Chem.* 15: 197-200.
1940a Bread baking test suggested for pastry flours. *Food Industries* 12: 52-54.
1940b A physical dough testing method applied to flour mill stream analyses. *The Northwestern Miller* (Production Number) Vol. 204, No. 5.

- Sherwood, R. C., and Bailey, C. H.
1926 Control of diastatic activity in wheat flour. II. Experiments with flour milled on a commercial scale. *Cereal Chem.* **3**: 163-182.
- Swanson, C. O.
1940 Factors which influence the physical properties of dough. I. Effects of autolysis on the characteristics of dough mixer curves. *Cereal Chem.* **17**: 689-700.
- Ziegler, E.
1938 The use of a purifier in experimental milling. *Cereal Chem.* **15**: 663-671.

SOME EFFECTS OF REWORKING FERMENTING DOUGHS

E. N. FRANK

International Milling Co., Minneapolis, Minnesota

(Read at the Annual Meeting, May 1941)

Freilich and Frey (1938) have demonstrated that remixing of fermented doughs will overcome a certain type of tightness or buckiness. They worked with straight doughs and for their purpose induced this condition by the addition of amounts of potassium bromate greatly in excess of those ordinarily employed in baking. Their work suggested that the same principle of treatment might be effective in improving the action of doughs which display natural buckiness.

Materials Used

Samples of hard wheats which produce doughs with natural buckiness are not infrequently encountered but the frequency varies from crop to crop.

Our laboratory was one of fifteen which collaborated in the testing of the 1940 crop spring wheat samples produced for the Northwest Crop Improvement Association. When the usual testing had been completed, we still had on hand a residual portion of the experimentally milled flour from each of the 24 wheats. Notations on our baking records indicated a wide range in the degrees of buckiness of the respective doughs. Two composites were therefore formed using eight of the flours. The four which had shown the most buckiness were blended to form a flour to which we gave the designation "bucky" and the four with least evidence of this characteristic to form the one designated as "nonbucky." The former showed by analysis 0.42% ash and 16.60% protein, and the latter 0.41% ash and 14.90% protein.

Experimental

The formula and procedure for the bakings to be described, unless otherwise indicated, were essentially those for the standard A. A. C. C. test. The doughs contained the following ingredients in addition to

those specified for the standard test: $1\frac{1}{2}\%$ sugar (additional to the specified $2\frac{1}{2}\%$), 0.1% ammonium carbonate, 2% lard, and 0.5% malted wheat flour. Doughs were mixed with a Hobart-Swanson machine and baked in the tall-form pans. All tests were made in sextuplicate. The two composite flours were first subjected to a simple mixing differential test. Results of this baking are shown in Table I.

TABLE I
EFFECT OF INITIAL MIXING PERIOD UPON LOAF VOLUME

Sample	Average loaf volume		Response
	2 min mixing (Schedule A)	3 min mixing (Schedule B)	
Bucky	810	693	-117
Nonbucky	847	879	+32

There was no marked difference of external appearance between the loaves from doughs which had been mixed for two minutes but there was a distinct difference in the three-minute loaves. Here the loaves from the bucky flour had a very rough break and shred, while those from the nonbucky one were externally smooth.

The two flours were next submitted to a series of baking tests in which remixing at various stages of fermentation was employed. The mixing schedules are shown in Table II. Average loaf-volume figures obtained are given in Table III. The data appearing in Tables I and III are shown graphically in Figure 1.

TABLE II
SCHEDULES OF REMIXING

Schedule	Initial mixing	Remixed in place of first punch	Remixed in place of second punch	Total mixing
	min	min	min	min
C	$2\frac{1}{2}$	$\frac{1}{2}$	0	3
D	$2\frac{1}{2}$	0	$\frac{1}{2}$	3
E	2	$\frac{1}{2}$	$\frac{1}{2}$	3

TABLE III
EFFECTS OF REMIXING UPON LOAF VOLUME

Sample	Schedule C	Schedule D	Schedule E
	cc	cc	cc
Bucky	835	888	933
Nonbucky	826	775	737

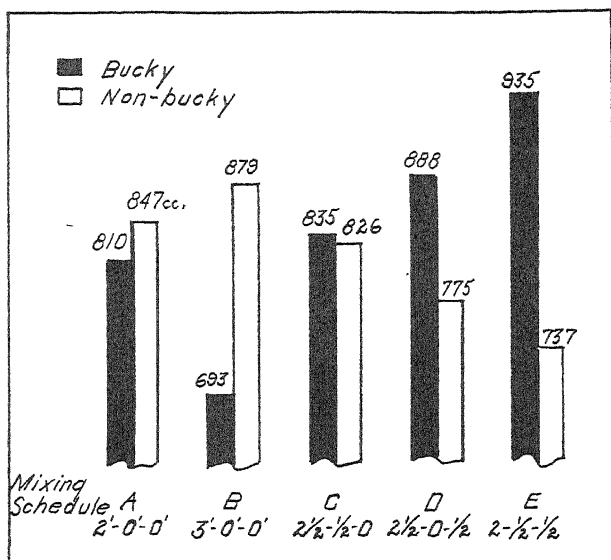


Fig 1 Effect of remixing of doughs on loaf volume

It is at once evident that every change in the mixing treatment which benefited the doughs of one flour handicapped those of the other. Deferment of a portion of the mixing treatment was of distinct benefit to the doughs from the bucky sample. For this flour the third minute of the initial mixing period was detrimental, but the same amount of mixing deferred to points of time when the doughs were in later stages of development was beneficial to loaf volume.

Further bakings were made using the three-minute initial mixing period and reworking the partially fermented doughs in a different manner. This was accomplished by passing them repeatedly through sheeting rolls to give the effect of braking. Preliminary experiments showed that a convenient and satisfactory stage at which to apply this treatment was at the time and in the place of the usual second punch. The number of passages through the rolls was increased by stages from 0 to 12 in multiples of 4. The effects upon loaf volume are indicated in Table IV and Figure 2.

Four passages through the brake caused an increase in loaf volume for both samples relative to their controls but with further increase of the braking effect, the volume of loaves from the bucky sample increased while that of the nonbucky one decreased. This is in general agreement with the effects of reworking by remixing.

TABLE IV
EFFECT OF BRAKING UPON LOAF VOLUME

Sample	Number of times through the brake			
	0	4	8	12
Bucky	688	726	758	773
Nonbucky	841	855	800	749

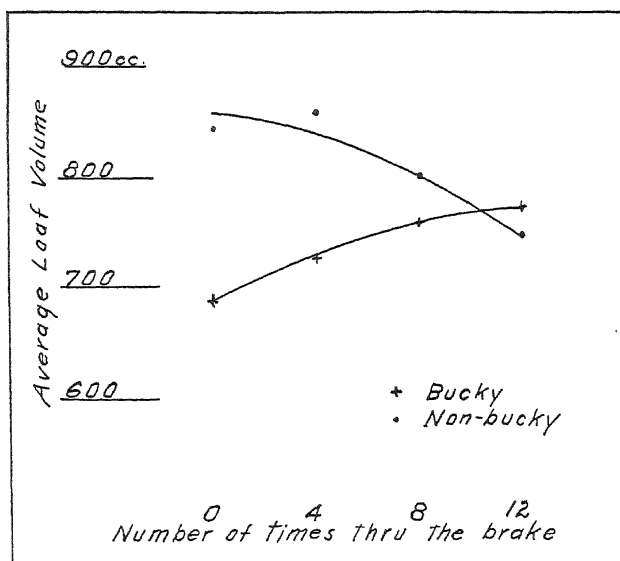


Fig 2. Effect of braking of doughs on loaf volume.

The break and fill-in of the loaves from the bucky sample became progressively smoother with increased application of the braking treatment, while those of the nonbucky one were smooth throughout the entire series.

Discussion

Sufficient experimental work to establish the immediate cause of natural buckiness has not been done. It appears to be due to a partial coagulation of the gluten proteins. It is favored in its development by increased oxidation due to prolonged initial mixing in contact with air and by increased hydrogen-ion concentration as fermentation proceeds.

It is retarded by mechanical reworking of the fermenting dough which probably prevents the formation of, or causes the destruction of, some type of association of particles. It is permanently removed by reworking the fully fermented dough.

The phenomenon may be due to thixotropism of the starch component although, if so, the observed effects of reworking should have been highly reversible. Markley (1937) has stated that: "A thixotropic system is one which is a gel when quiescent, but which becomes a sol upon the application of a shearing force, but again becomes a gel when allowed to return to the quiescent stage. . . . This process can be repeated many times."

The cause of natural buckiness in doughs from certain flours is suggested as an interesting subject for further research.

Summary

When judged by the results of a fixed straight-dough baking procedure employing a severe initial mixing treatment, there was a wide range in the degrees of buckiness of the respective doughs from a group of spring wheats grown in a single crop year.

Variations in the schedule and severity of mixing and of the mechanical working of fermenting doughs significantly affected their relative conditions of buckiness at the subsequent molding stage.

Under the conditions employed, remixing produced a larger effect than braking.

For a sample with an inherent tendency toward buckiness, extension of the initial period of mixing accentuated this characteristic; but deferment of a portion of the mixing treatment or more thorough reworking of the doughs at later stages of development reduced it.

Literature Cited

- Freilich, J., and Frey, C. N.
1938 Dough oxidation and mixing studies. II. *Cereal Chem.* **16**: 495-502.
Markley, M. C.
1937 Colloidal behavior of flour doughs. I. *Cereal Chem.* **14**: 434-436.

INTERPRETING EXPERIMENTAL MILLING DATA FROM A COMMERCIAL ASPECT

PERIE RUMOLD

Standard Milling Co , Kansas City, Kansas

(Read at the Annual Meeting, May 1941)

Experimental milling is recognized as an essential operation in many cereal laboratories. The purpose of the miniature mills is to provide flour for the experimental baker and the analyst for comparative tests with known standard varieties and standard commercial blends.

Experimental milling work is usually directed toward one of two main objectives: first, aiding plant breeders and agronomists in selecting new wheat varieties and, second, providing the commercial miller with advance milling data and the mill chemists with sufficient flour for preliminary test baking and analytical investigation. This information is valuable to the wheat purchasing department in selecting wheats that are satisfactory for future mill blends.

This discussion will deal principally with procedure and interpretation from a commercial and industrial viewpoint. The system used by some commercial mill laboratories will be outlined for the purpose of indicating the method of correlating the experimental mill results with actual commercial production.

The routine used in a mill laboratory will depend largely upon the ratio of wheat in storage to the output of the commercial mill. A system of experimental milling used where four or five months' supply of wheat is available may prove unsatisfactory where only a few weeks' supply of wheat is at one's disposal.

Milling tests should be made regularly, and it is very important to thoroughly clean out the mill before starting a day's run. One or two samples should be milled at the beginning and the results disregarded. Repeated tests in our laboratory indicate that dependable results are obtained only when beginning with the third sample. When time and help are limiting factors I think that far more reliable results can be obtained by milling four or five samples at a time than by milling one or two samples a day on several different days.

Some laboratories use the following method to determine the merits of new crops and to establish flour standards for the ensuing year. First each bin of wheat is selected according to protein, test weight, type of wheat, and point of origin. Then the wheat is thoroughly blended in such a manner that a sample of wheat taken at any time will be representative of the entire lot. Each bin is now

given a lot number and each lot is milled and the flour analyzed and baked. Various laboratories make from one to four millings on each lot of wheat. Additional milling tests can be made whenever the wheat is turned, to show the effect of storage and aging upon the milling and baking qualities of the wheat stocks.

Upon completion of this type of work one can make up blends from the various lots that will compare with those that have been used during the previous crop. After these combinations have been tested one can adopt a standard for each mix that is to be used as the basis of comparison between the old and new crop. This will determine the potentialities of the lots of wheat and wheat mixtures so that standards for the new crop can be established.

In designing an experimental milling system we have found that it is impractical to duplicate commercial milling results. Since the primary purpose of experimental milling work is to determine the comparative values of the different types of wheat a set procedure has been adopted.

One-thousand-gram samples are weighed and passed over a combination cleaner and scourer and the screenings are weighed to determine the cleaning loss. Then water is added and the wheat stirred until the moisture is uniformly distributed throughout the sample. The amount of water added depends on the moisture content and the type of wheat to be milled. The wheat is tempered in the late afternoon, placed in a closed metal container, and allowed to stand overnight.

The following day the tempered wheat is passed over the break rolls. Four or five breaks are used, depending on the cleanup desired. Two stands of break rolls are used with the following corrugations: one stand, fast roll, 12 corrugations to the inch, slow roll 20 corrugations to the inch and running dull to dull. The other stand has 24 corrugations to the inch on both rolls, running dull to dull. All corrugations are standard Dawson cut. Practically all of the endosperm is removed in the first three breaks and only a small amount of fine and inferior middlings are obtained from the fourth and fifth breaks.

The sizings and middlings stocks are now carefully reduced through a 64 GG sieve. At this stage as little flour is produced as possible. When the coarse middlings have been reduced through the 64 GG sieve the stock is sifted to reclassify the middlings and remove the flour that has been produced thus far. The classified stocks are now ground into flour, which takes from three to four reductions.

This system will yield approximately 625 grams of flour without grinding any stocks that contain large amounts of bran particles.

To increase the yield of flour we use the finer corrugated rolls on the last two breaks and further reduce the stocks not used in making the above-mentioned 625 grams of flour.

The sieve arrangement used on the breaks and coarse middlings reductions are, from top to bottom:

16 W
38 GG
64 GG
Blank

and on the reclassification and final reductions the sieves are stacked top to bottom:

<i>Hard wheat</i>	<i>Soft wheat</i>
64 GG	64 GG
8 XX	11 XX
11 XX	13 XX
Blank	Blank

The operator attempts to break the wheat in such a manner as to obtain a definite amount of products on each break, but there is apt to be a variation due to the type of wheat and the setting of the rolls. This depends upon the condition of the wheat before milling, atmospheric conditions, the type of wheat, and the judgment of the operator. The same procedure is used in reducing the middlings, and the cleanup depends entirely upon the skill of the operator.

Inasmuch as it is impossible to condition each type of wheat separately in the present-day arrangement of our commercial units, wheat or types of wheat that are to be mixed into a common blend are tempered or conditioned in the same manner. Without a definite system of conditioning one can lose sight of the milling characteristics if every sample is to be milled in a special manner to suit that particular lot of wheat.

The results obtained by this set procedure can be correlated with the results achieved in the commercial mill. Determining the ash content of the flour can be used to good advantage in deciding the best method of milling and to what degree the wheat must be tempered to obtain flour with the lowest ash content.

Assuming that one has equipped the laboratory with a satisfactory experimental mill and has a well established system of milling, it is necessary to provide a well trained and capable technician in order to obtain results that are reliable and comprehensive. Sometimes little thought is given to experimental mill work and its relation to commercial production. If insufficient capital is provided for proper facilities and personnel one cannot expect to derive maximum benefits from this phase of laboratory work.

A good technician should be able to judge milling values by observing such characteristics as potential extractions and flour yields, bran thickness, ability of the bran to hold together during the breaking operation, nature of middlings reduction, the character of the ground stock, and the time required for each sifting. All of these factors are very important to the mill superintendent and if properly interpreted and disclosed in an understandable manner they can serve as an important guide to the operative miller in the manufacture of a satisfactory and uniform flour. Close observation of the scalps from all the reductions in addition to the flour obtained will enable one to estimate the approximate yield of flour expected from a given lot of wheat.

It is very important to detect musty and sour odors, mold, heat and other forms of damage, foreign material of all kinds, cracked kernels, shriveled grain, and presence of smut and other abnormalities that are present in wheat samples from time to time. Occasionally a small amount of contaminated wheat that would escape the notice of an inexperienced operator could cause sufficient trouble to warrant the extra expense of employing a dependable operator. Frequent talks with the experimental miller tend to encourage efficient operation and help him to realize the importance of his work. I think that the quality of this type of work could be improved by including it as an educational and training course for the personnel in the wheat handling or wheat purchasing department.

I am sure most millers will agree that the results obtained from any experimental or commercial mill are greatly influenced by atmospheric conditions prevailing in the room during the milling process. At present very few experimental or commercial mills are equipped to control these conditions. The significance of uniform milling conditions is being recognized as one of the most important factors in both experimental and commercial mill work. From time to time this is brought to our attention by the invention and installation of equipment intended to artificially control or condition the air of flour mills.

The fact that experimental milling results vary with temperature and humidity has been emphasized by the work of Bayfield in recent years. This is further substantiated by experimental investigations in commercial mill laboratories. If milling results are to be strictly comparable they should be conducted under identical atmospheric conditions. There is a fairly close agreement among experimental millers that best milling results are obtained with a temperature of 78°-82°F and a relative humidity of 60%-65%, and if these conditions are maintained, milling results can be duplicated from day to day. It is well to keep in mind that the atmospheric conditions must be

constant if one wishes to make direct comparisons between commercial and experimental mill results. Millers and mill chemists agree that it is advisable to make comparisons with the same commercial mill unit, as it has been observed that results are not duplicated on different milling units.

It is readily possible to establish a basis for correlating experimental mill work with the operations of the commercial mill. We have found, however, that there will be occasional variations in the commercial mill that are not reflected on the same wheat blends when milled on the experimental mill. Therefore it is essential to check experimental mill results with the standard for any particular mix that has been established for experimental mill work. This procedure has been found acceptable to our production department, and its use has enabled us to inform that department of factors that are instrumental in maintaining efficient operation.

EXPERIMENTAL DURUM MILLING AND PROCESSING EQUIPMENT, WITH FURTHER QUALITY STUDIES. ON NORTH DAKOTA DURUM WHEATS¹

R. H. HARRIS and L. D. SIBBITT

North Dakota Agricultural Experiment Station, Fargo, North Dakota

(Received for publication August 27, 1941)

Harris and Knowles (1940) published data obtained from quality studies conducted upon the 1938 crop of North Dakota durum wheat. The wheats had been milled and the semolina processed into macaroni at the Dominion Grain Research Laboratory at Winnipeg, Canada, because of lack of standard equipment at the North Dakota Station.² The apparatus needed to carry on investigations interpretable in terms of commercial practice has since been purchased, and is described in some detail in this report. The present paper also contains data derived from a continuation of preliminary work reported previously, and comprises material obtained from the milling and processing of wheats produced in the crop years 1939 and 1940. Cooking-quality studies on the macaroni are planned for a later date, and will be published in due course.

Fifield (1934) and Fifield, Smith, and Hayes (1937) have discussed experimental equipment and methods for the manufacture of macaroni products, and have published results obtained from durum wheats

¹Published with the approval of the Director of the North Dakota Agricultural Experiment Station.

²L. D. Sibbitt, who was at that time experimental miller at the Dominion Grain Research Laboratory, was instrumental in performing the milling, processing, and quality evaluation of the macaroni.

grown in the hard red spring wheat region of the United States from 1932 to 1936. Binnington and Geddes (1936) described in detail experimental milling and processing apparatus for durum wheats, and presented a statistical basis for the evaluation of the results. Later Binnington and Geddes (1937) published data derived from a study of 34 samples of Canadian durum grown in 1934 and 1935. Significant differences in macaroni color and appearance were demonstrated among the samples. Further studies by Binnington and Geddes (1939) emphasized the point that macaroni quality cannot yet be predicted from any single analytical test applied to the wheat and that wheat carotene is valueless as an index of macaroni color, particularly for inter-varietal prediction.

Milling and Processing Equipment

The milling equipment consists of a two-stand Allis-Chalmers experimental mill, equipped with one bolter and a small-scale purifier. The mill is provided with 19th middlings cut rolls (6×6 -inch), one stand fitted with No. 16 and the other with No. 24 corrugations, both sets with $\frac{3}{4}$ -inch spiral and running dull to dull. A photograph of the mill is shown in Figure 1.

The purifier is a modified form of a Minneapolis commercial-type machine which was scaled down to laboratory dimensions by the removal of a screw conveyor in the base and the traveling brush attachment for cleaning the sieve. The sieve was also removed and a more suitable one (6 inches in working width) substituted to prevent portions of the sieve running bare when operating. The main sieve is divided into four portions consisting of sizes 50GG, 40GG, 34GG, and 28GG and is activated by a 3-horsepower electric motor. Three compartments were installed in the base of the purifier to receive the purified semolina and a suitable fan was housed at the rear top to furnish needed aspiration. The air current can be varied in intensity by levers placed outside the housing which regulate the size of the openings of the air channels from the fan chamber to the purifier proper. A dust collector was located in the basement of the building and the exhaust was piped to it. This machine is represented in Figure 2.

The macaroni processing apparatus consists of a mixer, kneader, and press mounted on a common table and driven by a $1\frac{1}{2}$ -horsepower motor. The set-up closely resembles the one described by Fifield (1934) and Binnington and Geddes (1936) and is shown in Figure 3. Limit switches stop the motor at either end of the press travel, and a small motor-driven propeller mounted on the jacket insures proper agitation of the oil bath surrounding the press chamber, which has a thermostatic temperature control.

The drying cabinet is modeled after the one described in detail by Binnington and Geddes (1936) with the exception that no refrigerating unit was included to aid in removing moisture from the air within the cabinet. A small vent in the system is opened gradually to permit the moisture-laden air to escape. The interior of the drier is lined with

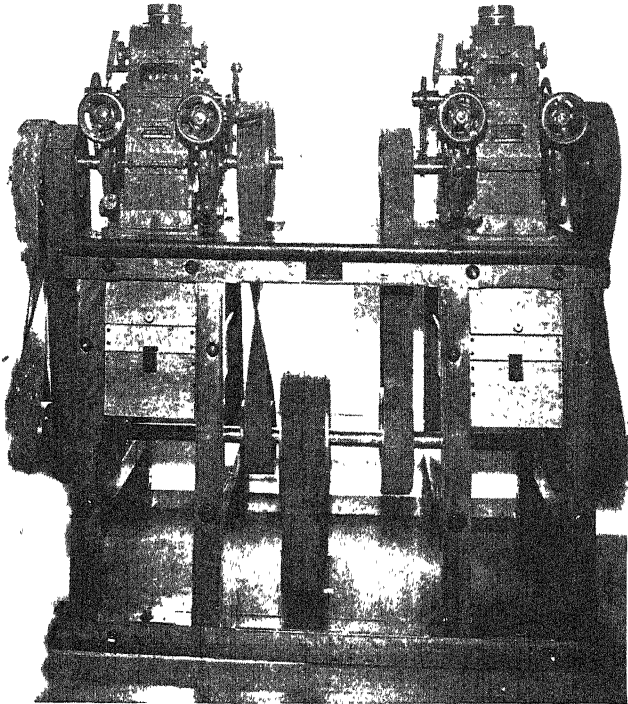


Fig 1. Experimental durum wheat mill.

thin sheet copper and a suitable set of louvres with handles projecting outside the cabinet was installed at either end of the drying chamber.

A Fenwall thermoregulator is used to control the temperature through a 192-watt heater. The control bulb of this instrument, placed in the air-flow coming directly from the humidifying and heating chambers, proved very satisfactory for holding the temperature at a constant level during drying.

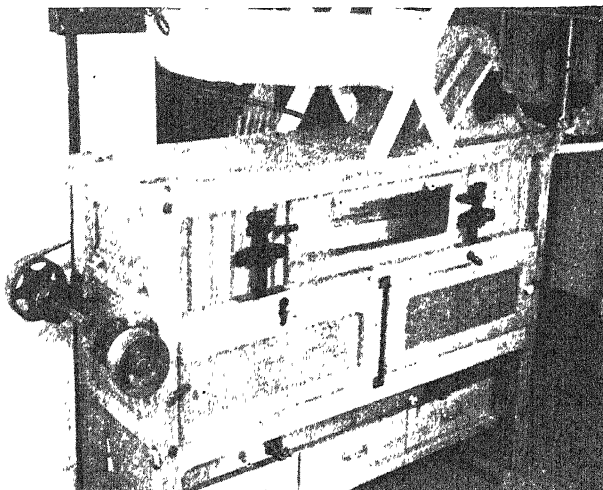


Fig 2. Experimental purifier used in durum wheat milling.

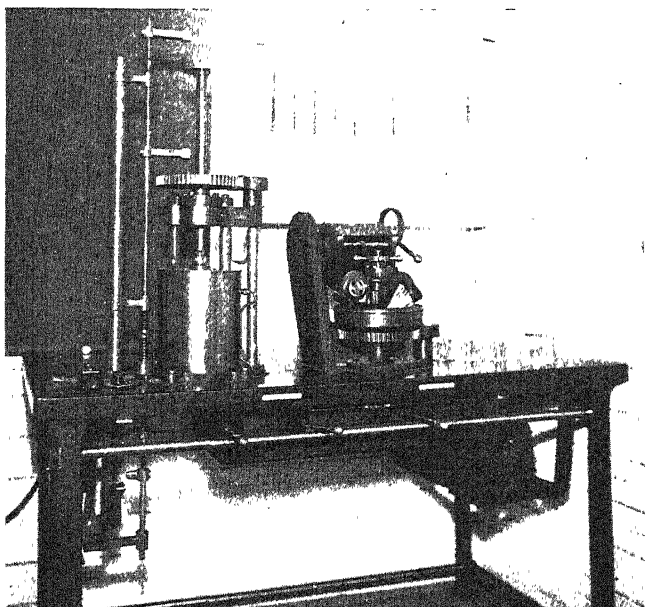


Fig. 3. Experimental macaroni processing unit.

A variable resistance boiler of the type described by Binnington and Geddes is employed as a source of humidity. A vacuum-tube relay is used to control the input of current to the boiler. This relay is connected to the wet-bulb pen of the Taylor temperature recorder, and functions by permitting the electric current to pass directly to the carbon electrodes of the boiler when it is necessary to raise the humidity of the cabinet. When the cabinet humidity has reached the desired point the current is shunted to the boiler through a resistance lamp bank. Under these conditions sufficient heat is generated by the reduced current to maintain the water in the boiler just below the boiling point. Suitable charts were constructed to control the relative humidity at the proper levels during the drying period. The drier is shown in Figure 4.

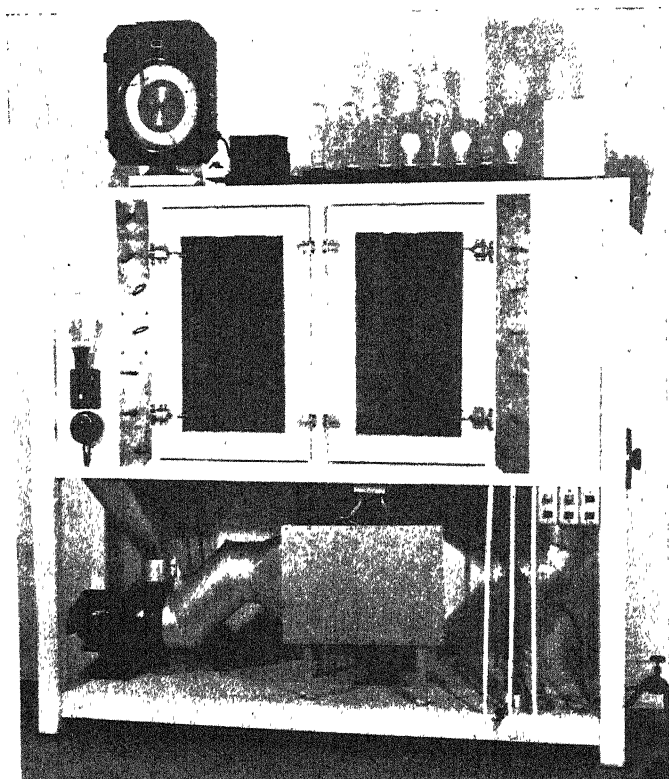


Fig. 4. Experimental drying cabinet with temperature and humidity control equipment. Relay and lamp resistance bank shown at top of drier.

Milling Technique

Cleaned wheat is used for the milling tests and the milling samples are weighed to give 3,000 g of wheat on a 13.5% moisture basis. Sixteen hours before milling, sufficient water is added to bring the moisture content to 13.5%. At the end of the conditioning period the sample is scoured and the moisture raised to 15.5% 90 minutes prior to milling. The mill laboratory temperature is held at approximately 70°F and the relative humidity at about 60%. The milling is conducted according to the flow sheet shown in Figure 5. Ten breaks are employed and all

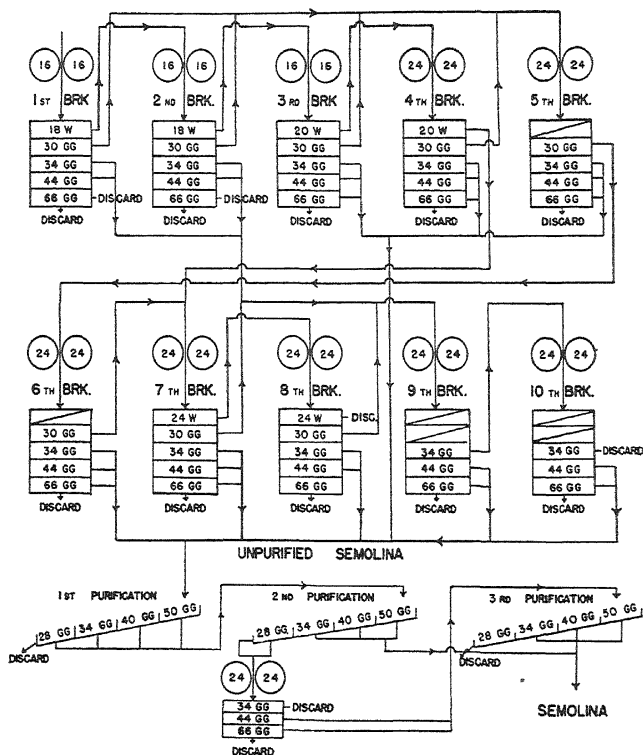


Fig. 5. Experimental flow sheet for durum milling.

stock after the second break, passing through a No. 30GG and retained on a No. 66GG, is bulked together and weighed as unpurified semolina. This material is then passed through a purifier in a thin stream with a light air flow. The semolina passing through the sieves of the purifier

is bulked together and passed through a second time, the tailings being discarded. In this second purification the maximum air flow obtainable is used. The throughs from Nos. 50, 40, and 34GG are composited. The tailings and the throughs from No. 28GG are reground and bolted, and the material remaining on Nos. 44 and 66GG is given a third purification. The throughs from Nos. 50, 40, and 34GG are thoroughly mixed with the material taken off in the second purification and weighed as purified semolina.

Processing Technique

The major operations included in macaroni processing are mixing, kneading, pressing, fanning, and drying. The laboratory is maintained at 55% to 60% relative humidity while the processing is being

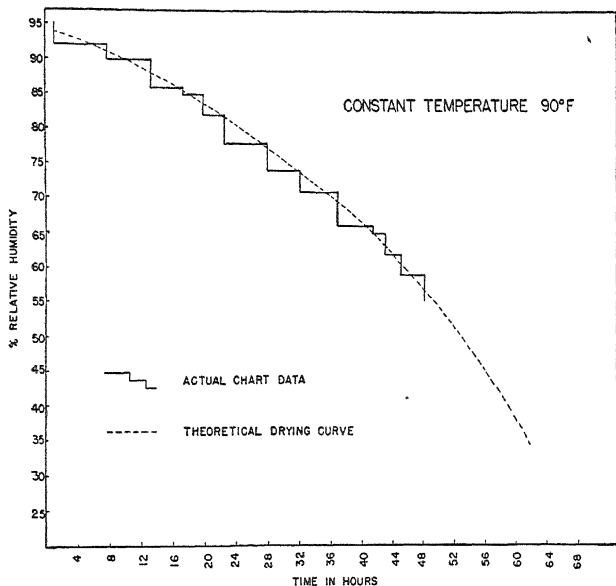


Fig. 6. Time-humidity gradient used in the experimental drying of macaroni.

done. The press temperature is held at 92°F. A special macaroni die is used which is substantially thicker than the customary experimental dies and has its center rod held firmly by three knife-edge supports instead of one, as is usual in experimental dies. This arrangement prevents possible displacement of the center rod during pressing with resultant variations in macaroni wall thickness. In processing, 600 g

of semolina on a 13.5% moisture basis is used. Sufficient water is added to form a stiff dough, the semolina and water are then mixed, and kneaded to optimum consistency. The dough is permitted to rest for ten minutes at press temperature before being pressed into macaroni. The 30-inch lengths of macaroni are suspended over wooden rods and surface-dried at room temperature in an air current from a fan. The material is then placed in the drying cabinet where it is sweated for a minimum period of one hour at 90°F and 95% relative humidity. The drying of the macaroni is performed in the cabinet, which is fitted with devices for accurately and automatically controlling the temperature and humidity.

The apparatus and methods employed closely resemble those used in commercial semolina and macaroni manufacture. Drying is done at a constant temperature and under a falling humidity gradient as represented in Figure 6. The visual color score of the macaroni was determined under a mercury fluorescent lamp.

Material

Thirty-two samples of durum wheat grown at Fargo and Langdon³ were experimentally milled and the semolina processed by the equipment described, using the standardized techniques developed by Binnington and Geddes (1936). The moisture and protein contents of the wheat and semolina were determined, as well as the number of specks per ten square inches of semolina. It is somewhat unfortunate that only wheat of the 1940 crop was available at Langdon as the district is noted for high-quality durum production, but the quality suffered severely from damage caused by fungus attacks and other injurious factors which were favored by unfavorable weather conditions during July, August, and September, as pointed out by Harris and Sibbitt (1941). The results obtained on the Langdon wheats are markedly lower as a result of the effect of these conditions. Fargo lies southeast of the area of best-quality durum production for macaroni purposes, and the plots at this station escaped substantial damage in 1940.

Discussion of Data

In Table I are shown the description of the wheats with associated protein and milling data. A number of varieties were included in these series of wheats to cover fairly well the field of quality. Several of the varieties, such as Pentad, Golden Ball, and Monad, are well known to be unsuitable for the production of quality macaroni. Other varieties are under test with the view of introducing them for general production

³ The varieties from this Station were grown in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

TABLE I

WHEAT DESCRIPTION, PROTEIN, AND MILLING DATA ON DURUM
WHEATS GROWN IN 1939 AND 1940

(Results arranged in order of increasing wheat protein content within stations)

Lab. No	Variety	Test wt per bu.	Unofficial grade	Kernel damage			Protein (N X 5.7) ¹		Semolina yield	
				Light	Heavy	Total	Wheat	Semo- lina	Unpur- ified	Pur- ified
		lbs		%	%	%	%	%	%	%
FARGO 1939										
39-643	Golden Ball	62.1	1 HAD ²	—	—	—	15.6	13.3	65.7	43.0
646	Kubanka 314	63.1	1 HAD	—	—	—	15.4	13.7	65.4	43.3
644	Pentad	63.8	RD	—	—	—	14.3	12.1	65.7	43.1
649	Ld 9 X Min	64.1	1 HAD	—	—	—	14.3	13.0	65.0	45.6
648	Ld 34	63.7	1 HAD	—	—	—	14.2	12.8	66.2	45.3
647	Kubanka 74	63.6	1 HAD	—	—	—	13.9	12.6	65.9	44.4
641	Kubanka	63.8	1 HAD	—	—	—	13.8	12.4	65.3	43.3
640	Mindum	64.0	1 HAD	—	—	—	13.5	12.5	66.2	44.4
642	Monad	63.9	1 HAD	—	—	—	13.5	11.6	66.6	45.1
645	Kubanka 49	64.4	1 HAD	—	—	—	13.1	12.0	64.4	43.3
FARGO 1940										
40-900	Ld 134	64.0	1 HAD	Some broken kernels			14.4	12.9	64.8	43.6
897	Ld 102	63.9	1 HAD	Some black point			14.2	12.7	65.8	43.3
894	Kubanka	63.9	1 HAD				14.1	13.1	66.5	47.7
898	Ld 104	64.6	1 HAD	Some black point			14.0	12.6	67.1	43.7
895	Pentad	64.0	RD	Some black point			13.9	12.5	65.9	44.9
899	Ld 111	64.5	1 HAD	Trace black point			13.9	12.2	65.0	42.5
896	Ld 34	64.5	1 HAD	Some black point			13.8	12.3	66.6	46.2
902	Kubanka 314	64.2	1 HAD				13.5	12.1	65.7	44.6
893	Mindum	63.8	1 HAD	Some black point			13.4	12.3	65.6	44.6
901	Kubanka 49	64.5	1 HAD				13.2	12.0	64.1	42.8
LANGDON 1940										
40-876	Ld 101	60.8	4 HAD	3	10	13	15.5	14.3	64.4	43.3
873	Kubanka	61.4	1 HAD	5	—	5	15.2	14.1	65.0	45.2
882	Ld 134	62.1	2 HAD	3	3	6	14.9	13.5	64.8	44.7
875	Ld 34	62.2	3 HAD	3	5	8	14.5	12.9	65.3	43.1
877	Ld 102	61.9	4 HAD	2	10	12	14.5	12.9	63.3	43.9
881	Ld 133	62.7	3 HAD	5	5	10	14.3	13.1	64.8	44.5
879	Ld 105	62.9	3 HAD	4	6	10	14.2	12.8	66.3	46.0
874	Monad	61.4	1 HAD	6	2	8	14.1	12.6	64.3	43.8
883	RL 1317	62.3	2 HAD	5	3	8	14.1	12.8	66.8	45.0
878	Ld 104	62.7	3 HAD	6	7	13	14.1	12.8	64.1	43.4
872	Mindum	62.0	3 HAD	12	7	19	14.0	12.6	65.2	44.9
880	Ld 111	62.1	5 HAD	5	12	17	14.0	12.4	65.3	46.1

¹ Data on 13.5% moisture basis.² HAD = Hard Amber Durum, RD = Red Durum.

in the durum area of the state if found superior in agronomic and macaroni-quality characteristics to the durums that are now being grown. These varieties are denoted by numbers as they have not yet been named. Mindum and Kubanka have proved to be fairly satisfactory in agronomic and quality factors and are the varieties which are at present in general production. The test weight per bushel varies from 64.6 to 60.8 lbs. The grade varies from No. 1 Hard Amber Durum to No. 5 Hard Amber Durum, the lower grades being found without exception in the Langdon series.

The effect of fungus infections in 1940 is evident in the damaged-kernel percentages. The light kernel damage classification contained the kernels which showed tip discoloration without visible damage in the crease or other parts of the kernel. The evidence of this form of damage can be almost entirely removed by rubbing the infected part of the kernel. The heavy-damage classification comprised kernels with more of the surface, including the crease, showing injury. Total kernel damage is the sum of light and heavy kernel damage. Some variability in wheat protein among the samples is evident, especially in the 1939 Fargo series. These differences must be ascribed to differential varietal responses to environmental conditions of soil and climate. The semolina protein is less variable. The yields of unpurified and purified semolina are both given in the table, the latter values of course being much lower than the former as a result of removal of bran and fibrous material during purification. The number of specks was greatly reduced by purification, while the color was correspondingly improved.

Table II presents the absorption and quality ratings of the semolina and macaroni. The number of specks in the semolina is greatly increased in the 1940 Langdon samples, but on the other hand a noticeable amount of damage on the 1940 Fargo wheats was not reflected in increased semolina speckiness. This was no doubt owing to the fact that the damage had not penetrated through the bran to damage markedly the milling quality of the kernel. The absorptions are fairly consistent and do not appear to have been greatly affected by the unfavorable weather conditions at Langdon. A marked range in color score of macaroni is noticeable, varying from 2.0 to 9.0 at Fargo, 1939; from 2.0 to 8.0 for Fargo, 1940; and from 2.0 to 5.0 for Langdon, 1940. In addition, the Langdon samples were more or less brown and pale in color and would be unsatisfactory for commercial grade long goods.

The varietal color scores are presented in Figures 7 to 9. In each figure the data obtained from one series is represented. Figure 7 presents the Fargo 1939 color scores, while Figure 8 presents similar results for 1940. The Langdon results for 1940 are presented in Figure 9. It will be noticed that Mindum was first in the group in color rating

TABLE II

ABSORPTION AND QUALITY RATINGS OF THE SEMOLINA AND MACARONI

(Results arranged in order of increasing macaroni color score within stations)

Lab No	Variety	Semolina			Visual color score of macaroni, perfect score 10
		Specks per 10 sq. in.	Rating within stations	Absorption %	
FARGO, 1939					
39-642	Monad	50	7	27.2	2.0 Pk
643	Golden Ball	32	4	28.1	2.0 Pk
644	Pentad	78	8	28.0	2.0 Pk
641	Kubanka	44	6	27.4	5.0
645	Kubanka 49	30	3	28.2	7.0
646	Kubanka 314	20	1	28.1	7.0
648	Ld 34	28	2	28.1	8.0
647	Kubanka 74	32	4	28.0	9.0
649	Ld 9 X Min	34	5	28.1	9.0
640	Mindum	30	3	27.2	9.0
FARGO, 1940					
40-895	Pentad	42	9	27.2	2.0 Br
901	Kubanka 49	26	7	27.4	5.0
902	Kubanka 314	22	6	27.4	5.0
894	Kubanka	12	2	27.7	6.0
896	Ld 34	10	1	27.5	6.0
897	Ld 102	18	4	27.2	6.0
900	Ld 134	30	8	27.7	6.0
893	Mindum	22	6	27.4	8.0
898	Ld 104	20	5	27.6	8.0
899	Ld 111	16	3	27.8	8.0
LANGDON, 1940					
40-874	Monad	32	1	28.8	2.0 Br
875	Ld 34	54	3	28.0	4.0 Br
877	Ld 102	102	8	28.0	4.0 Br
881	Ld 1333	64	4	27.8	4.0 Br
882	Ld 134	102	8	28.1	4.0 Br
883	RL 1317	64	4	27.8	4.0 Br
872	Mindum	94	7	27.3	4.0 P
876	Ld 101	82	6	28.1	4.0 P
879	Ld 105	70	5	28.0	4.0 P
880	Ld 111	126	10	28.0	4.0 P
878	Ld 104	112	9	28.1	4.5 Br
873	Kubanka	42	2	28.7	5.0

¹ Br = brown, Pk = pink, P = pale.

at Fargo in both years. At the Langdon station, on the other hand, Kubanka was first in 1940. Ld 104 was also high, being equal to Mindum at Fargo and second to Kubanka at Langdon, while Ld 111 was in the first classification at Fargo and third in Langdon. These

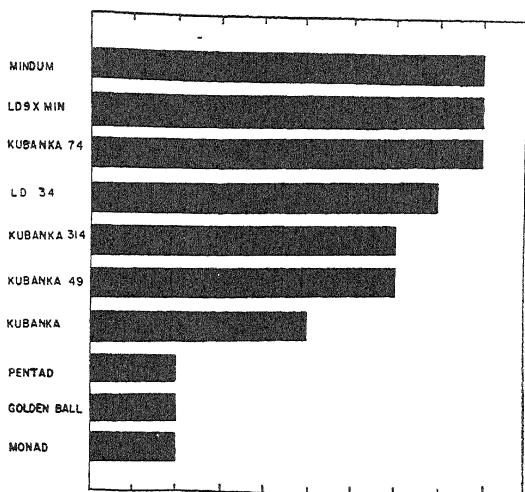


Fig. 7. Visual color score of macaroni processed from durum wheat grown at Fargo in 1939.

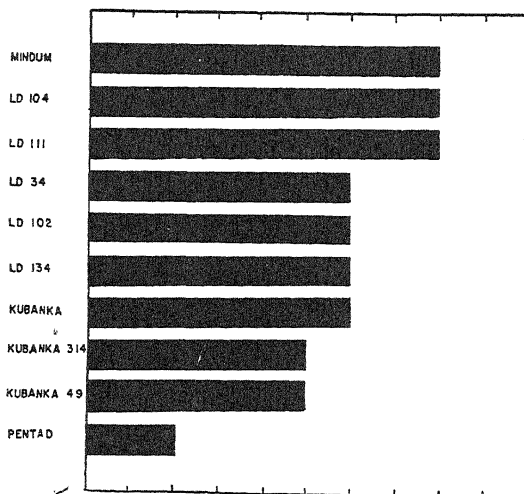


Fig. 8. Visual color score of macaroni processed from durum wheat grown at Fargo in 1940.

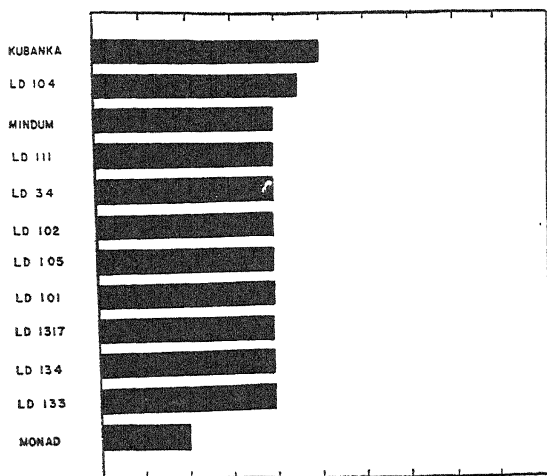


Fig. 9. Visual color score of macaroni processed from durum wheat grown at Langdon in 1940.

two varieties were not included in the 1939 Fargo samples. Ld 34, which was second highest in Fargo in 1938 (Harris and Knowles, 1940), was second in Fargo in 1939 and also in the second group in 1940. Monad, Golden Ball, and Pentad were at the foot of the list wherever grown in 1938, 1939, and 1940. This result is in agreement with their accepted quality rating. No doubt the results obtained from the 1940 samples grown at Langdon have been vitiated as a result of wheat damage. It is also probable that the Fargo values were also adversely affected to some extent, although the visual damage was very much smaller.

In Table III are shown the correlation coefficients calculated between several pertinent variables. Wheat protein and semolina pro-

TABLE III
CORRELATION COEFFICIENTS COMPUTED FROM THE DATA
(Significant coefficients in bold type)

Variables correlated		Correlation coefficients	Probability <i>P</i>
<i>x</i>	<i>y</i>		
Wheat protein, %	Semolina protein, %	+.8797	<.0001
Wheat protein, %	Semolina absorption, %	+.5317	.0060
Test weight, lbs per bu	Semolina yield (purified), %	+.0021	>.5485
Semolina yield (unpurified), %	Semolina yield (purified), %	+.4510	.0178
Semolina specks	Visual macaroni score	-.4910	.0105
Test weight, lbs per bu	Semolina yield (unpurified), %	+.3935	.0365

tein are very significantly correlated as would be expected by anyone familiar with durum wheat technology. Wheat protein and semolina absorption are also significantly and positively correlated. This is an interesting relationship, as it shows that semolina milled from relatively high-protein durum wheat will take more water to produce a dough of standard consistency. Test weight per bushel was not related to purified semolina yield in the results obtained in this study but was significantly related to yield of unpurified semolina. It is probable that the relatively large number of durum varieties included in the study materially decreased the correlation between test weight and semolina yield. The yield of purified semolina was positively correlated with the yield of unpurified semolina. This relationship, however, was not of sufficient magnitude to permit the prediction of one variable from the knowledge of another. The relationship between the semolina speckiness and visual macaroni score was also determined, although these were subjective measurements. A significant negative correlation was found between these variables, but the magnitude of this correlation was not sufficiently great to be of marked utility in predicting one variable from a knowledge of the other.

Summary and Conclusions

Milling and processing equipment for durum wheat at the North Dakota Experiment Station has been described in some detail, and the techniques employed in the quality evaluation of 32 samples of durum wheat outlined.

The equipment consists of a two-stand Allis-Chalmers experimental mill fitted with suitable rolls, a macaroni processing unit comprising a mixer, kneader, and press, the latter fitted with a device for accurately controlling the press temperature, and a drying cabinet equipped with accessories which enable a time-humidity gradient to be established during the drying period.

A description of the flow sheet used in durum milling is included, as well as a time-humidity gradient chart showing the various relative humidities obtained during the drying period.

Thirty-two samples of durum wheat grown at Langdon and Fargo in 1939 and 1940 were milled and processed by the equipment and methods described in this paper. These samples included varieties which have been shown to have satisfactory quality performance as well as new varieties now under examination with the purpose of possible release later for general production, provided the agronomic and macaroni-making qualities are satisfactory. A few undesirable varieties were also examined to obtain data in comparison with the other wheat studied.

The results of the 1940 wheats grown at Langdon were markedly affected by damage caused by unfavorable weather conditions preceding and during harvest. Injury from various forms of blight, bacterial infections, weathering, etc. was reflected in kernel discoloration, semolina speckiness, and visual color score of macaroni.

Mindum was in the first group for macaroni color in Fargo for both seasons, while Kubanka was among the first in 1940. Ld 104 was another variety which had relatively high macaroni color scores at both stations while the second new wheat, Ld 111, was next. Both wheats showed excellent promise.

Wheat and semolina protein were highly correlated, with wheat protein being related to a lesser degree with semolina absorption. Test weight per bushel was positively related to yield of unpurified semolina but not with yield of purified semolina. Semolina speckiness was inversely related to macaroni color scores.

Literature Cited

- Binnington, D. S., and Geddes, W. F.
1936 Experimental durum milling and macaroni-making technique. *Cereal Chem.* 13: 497-521.
1937 The relative macaroni-making quality of a number of durum wheat varieties. *Cereal Chem.* 14: 293-304.
1939 Further studies upon the relative macaroni-making quality of a number of durum wheat varieties. *Cereal Chem.* 16: 384-392.
- Finfield, C. C.
1934 Experimental equipment for the manufacture of alimentary pastes. *Cereal Chem.* 11: 330-334.
- Smith, G. S., and Hayes, J. F.
1937 Quality in durum wheats and a method for testing small samples. *Cereal Chem.* 14: 661-673.
- Harris, R. H., and Knowles, Darline
1940 Quality studies on North Dakota durum wheats (1938 crop). *Cereal Chem.* 17: 480-490.
- and Sibbitt, L. D.
1941 Blight and other damage of wheat in 1940. (1) Effect on macaroni-making quality. *N. D. Agr. Exp. Sta. Bul.* 296.

THE QUALITY OF NORTH DAKOTA DURUM WHEAT AS AFFECTED BY BLIGHT AND OTHER FORMS OF DAMAGE IN 1940¹

R. H. HARRIS and L. D. SIBBITT²

North Dakota Agricultural Experiment Station, Fargo, North Dakota

(Received for publication September 26, 1941)

During the ripening and harvest season of 1940 the weather conditions in the North Dakota durum-growing region were very favorable for the growth of various microorganisms upon durum wheat, while incipient sprouting, weathering, and similar factors also injuriously affected durum quality. These various forms of damage combined to decrease the grade of the wheat and, in many instances, prevented Federal loans from being made because the grade fell below the minimum allowable for loan purposes. Little information is available in the literature regarding the effects of damage by these causes upon the quality of macaroni.

One of the chief sources of worry to the durum trade in respect to the effects of unfavorable weather upon durum quality is the discoloration of more or less of the surface of the kernel caused by the fungus *Helmenihosporium*. This condition is known as "black point" in common parlance. The development of this fungus is favored by the accumulation and retention of moisture at the germ end of the kernel. Buyers of durum wheat have been convinced for some time that "black point" infection in durum wheat has caused substantial damage to quality. The tendency is for the commercial grain buyer to avoid any section in which wheat damage has been reported. The difficulty occasioned by the infection lies in effecting a clean separation in milling; the semolina contains black specks which in turn exert a degrading influence upon the macaroni. Some chemists have thought that a small degree of contamination is permissible, without injury to semolina color, but extreme care would have to be taken regarding the quantity allowed in the mix as well as in the degree of damage of individual kernels. Available information has indicated that the presence of very small proportions of completely discolored kernels is much more serious than partly damaged kernels. There is also evidence that black-tipped and black-creased kernels are about equally effective in regard to semolina color.

Brentzel (1941) found two types of damage present in the 1940 North Dakota durum wheat crop. These were respectively classified

¹Published with the approval of the Director of the North Dakota Agricultural Experiment Station.

²No seniority in authorship is implied

as "black point" and "other types." Black point was discovered in many samples, and consisted of two forms of similar appearance which could not be separated by visual inspection. Cultural and microscopic examinations, however, showed that most of the black point was caused by species of the fungus *Alternaria*, while a smaller portion resulted from the presence of *Helmenthosporium sativum*. Kernels affected by the former were often plump and heavier than unaffected seeds. There was no noticeable shriveling caused by this fungus, but germination was somewhat impaired. Kernels attacked by *Helmenthosporium*, on the other hand, were somewhat shriveled.

The "other types" comprised all defects except black point. A little scab (*Gibberella*) was present with some bacterial infection and a number of molds. Damage due to weathering, sprouting, shriveling, etc., was also present.

To evaluate the effects of this damage upon macaroni-making quality Harris and Sibbitt (1941) conducted a preliminary investigation upon a small number of samples which were available, using a modified form of the standardized procedures described by Binnington and Geddes (1936) with the exception that a scientifically controlled drying cabinet was not used for drying the macaroni. A second small series of samples was prepared from blends of light and heavily damaged wheat mixed with the same wheat with the damage removed. Because of the time required to separate the wheat into the various portions it was not possible to procure sufficient grain for the standard durum milling and processing technique, and accordingly the micro method described by Fifield, Smith, and Hays (1937) was employed. This method requires only 100 g of wheat, and consists essentially in milling a relatively small quantity of wheat according to the methods used in milling the customary-sized sample with slight modifications, mixing to a stiff dough, kneading by repeatedly passing the dough through a pair of manually operated steel rolls, pressing, and drying. A hydraulic press is used for the pressing and the disks are then dried. This method gives results which compare favorably, in respect to color, with values yielded by the standard technique.

The results obtained from the investigation showed that the chief effect of the damage was to increase the number of specks in the semolina and to decrease the semolina and macaroni color. The yield of semolina was also decreased. Heavily damaged kernels which showed extensive surface and crease injury had the greatest effect in decreasing quality, but light injury, visible only at the tip and removable by rubbing, if present in sufficient quantity, increased the speckiness and decreased the color ratings.

The conclusions derived from this tentative study convinced the authors that a further investigation should be made, using a larger number of samples which would cover a wider range of injury. It was also felt that additional information could be obtained by employing the standardized technique described by Harris and Sibbitt (1942) since an experimental drier with controlled temperature and humidity was now available. Suitable assistance was secured from the WPA to separate the wheat into the different classifications desired in the investigation and the following study undertaken.

Experimental Material and Methods

A large sample of damaged durum wheat was obtained from the territory in which wheat injury was prevalent. This sample graded (unofficially) No. 5 Hard Amber Durum and contained 28% damaged kernels. As in the previous investigation the total kernel injury was divided into light and heavy damage for the purposes of the investigation. All injured kernels were calculated as per cent by weight of the total. The classification of damage was done by two operators who worked under the same source and intensity of illumination throughout the project. Before making up the blends, the various separations were thoroughly reexamined by experienced operators, and any kernels that did not appear to be properly placed were reclassified. It is felt that the separations were as truly representative of the indicated degree of injury as it was possible to obtain. The various proportions of damaged wheat included in the blends were chosen to yield as much information as possible upon the effect of the amount of damage upon the quality of the wheat, semolina, and macaroni. The percentages of infected wheat were accordingly varied from 5% to 75% by weight. These blends were made with a good-quality durum grown in 1939. The various samples were thoroughly mixed before sampling and milling.

The different lots of blended wheat were analyzed for moisture and protein content. Test weight per bushel and grade were determined and 3,000 g of the wheat were taken for milling into semolina. The milling was done on a double-stand Allis-Chalmers experimental mill fitted with Allis-Chalmers 19th middlings cut rolls running dull to dull with $\frac{3}{4}$ -inch spiral. One pair of rolls contained 16 while the other had 24 corrugations per linear inch.

The flow sheet used corresponds to the one illustrated by Harris and Sibbitt (1942).

Discussion

A description of the blends used in this investigation with associated data are shown in Table I. The samples are arranged in order of increasing macaroni color score. The test weight and grade tend to decrease with an increase in percentage of damaged wheat included in the blend, particularly if the damage was heavy. The test weight fell from 62.2 to 58.8 lbs per bushel when 50% of heavily damaged kernels was included in the mix, while the grade decreased from No. 1 Hard Amber Durum to Sample Grade Durum. The protein of wheat and semolina tends to increase with addition of damaged wheat, regardless

TABLE I
WHEAT DESCRIPTION, PROTEIN, AND MILLING DATA ON DAMAGED DURUM WHEATS
(Results arranged in order of increasing macaroni color score)

Lab. No.	Description of blend ¹	Test wt. per bu.	Un-official grade	Protein (N \times 5.7) ²		Semolina yield ²	
				Wheat	Semolina	Unpurified	Purified
		lbs		%	%	%	%
40-908	50% heavy	58.8	SGD	14.6	12.9	64.3	45.5
909	25% heavy	60.4	SGHAD	13.8	12.6	64.6	45.4
910	10% heavy	61.7	4 HAD	13.5	12.2	65.2	45.7
906	Original (16% light, 12% heavy)	61.1	5 HAD	14.9	13.0	65.9	45.3
912	75% light	61.5	1 HAD	14.2	12.8	64.9	46.0
911	5% heavy	61.9	3 HAD	13.4	12.1	64.9	44.9
913	50% light	61.7	1 HAD	13.9	12.6	66.0	45.2
914	25% light	61.8	1 HAD	13.6	12.4	64.1	44.5
915	10% light	62.0	1 HAD	13.2	12.1	63.3	43.6
916	5% light	62.1	1 HAD	13.2	12.0	64.0	44.6
870	No visible damage	62.9	1 HAD	13.1	12.3	65.9	46.8

¹ Blends made with 40-870 plus indicated percentage of damaged wheat

² Data on 13.5% moisture basis.

of whether the damage was light or heavy. This was caused by the higher percentage of protein in the damaged wheat. No consistent trend in semolina yield was evident when the proportion of damaged wheat in the blend was changed.

In Table II are shown the speck count of the semolina, semolina quality rating, absorption, and visual color score. The increase in number of specks per ten square inches with increase in amount of damage is very clearly shown. There was little difference in absorption among the semolinas manufactured from the various blends, and apparently this property was not affected by wheat damage of this nature. The effect of percentage of damaged kernels upon macaroni color is very evident in the data, especially in the case of heavy kernel damage.

TABLE II
ABSORPTION AND QUALITY RATINGS OF THE SEMOLINA AND MACARONI

Lab. No	Description of blend ¹	Semolina ²			Visual color score of macaroni ³ (perfect score 10)
		Specks per 10 sq. in.	Rating	Absorption %	
40-908	50% heavy	220	10	27.6	1.0 br mottled and specky
909	25% heavy	134	9	28.1	3.0 br mottled and specky
910	10% heavy	106	7	27.6	4.0 br mottled and specky
906	Original (16% light, 12% heavy)	130	8	28.1	4.5 br mottled
912	75% light	64	6	28.1	5.0 sl br mottled
911	5% heavy	50	4	28.0	8.0 mottled
913	50% light	60	5	28.0	8.0 mottled
914	25% light	40	3	28.2	8.0 mottled
915	10% light	16	1	28.3	9.0 sl mottled
916	5% light	16	1	28.2	9.0
870	No visible damage	28	2	27.7	9.0

¹ Blends made with 40-870 plus indicated percentage of damaged wheat.

² Data on 13.5% moisture basis.

³ Br = brown; Sl = slightly.

In Figure 1 the effects of the different percentages of light and heavy damage upon test weight, semolina speckiness, and macaroni color score are shown graphically. The influence of the amount of heavy damage upon these three factors is strikingly brought out and illustrates the care required to control rigidly the proportion of heavily damaged kernels allowed in a durum mix. Five per cent of such damage seriously lowered the color of the macaroni, while 10% was extremely detrimental to macaroni color as well as to semolina speckiness. It also adversely affected the test weight per bushel. The inclusion of 50% of heavily damaged kernels decreased the test weight to less than 59 lbs, increased the number of specks per ten square inches to well over 200, and reduced the macaroni color score to a very low value. While no commercial mill would consider using a mix containing such a high proportion of this form of damage, the data are useful in showing the extremely adverse effects of the blend.

The light kernel damage had much less effect upon the three factors represented. Test weight, while slightly reduced, did not fall below 61 lbs even when 75% of light damage was present. The number of semolina specks also was increased only very slightly. The greatest effect of this form of damage was found to be upon the macaroni color, but when the percentage of damaged kernels was below 10% there was apparently no effect and 25% did not seriously degrade the color. When 50% was present, however, the color commenced to fall off

rapidly, and when 75% was reached the macaroni was decidedly inferior.

Figure 2 represents the effect of the various percentages of damaged

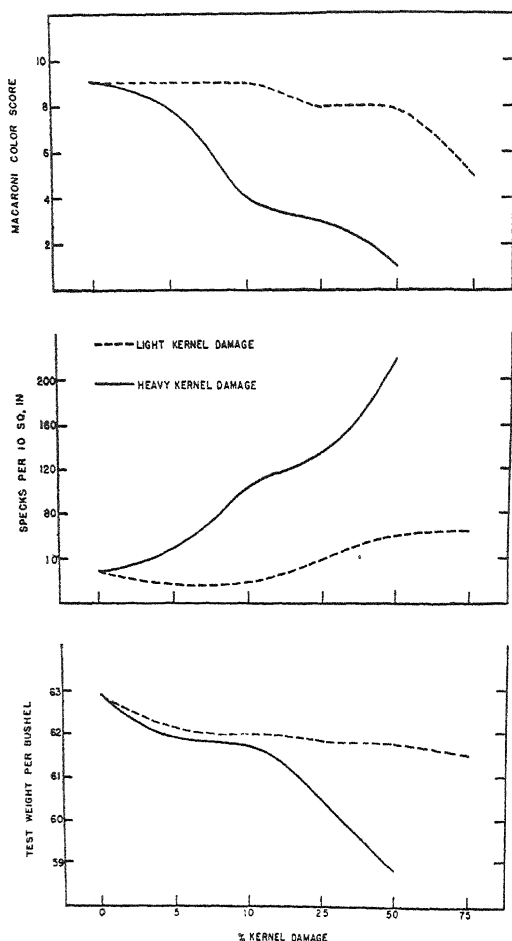


Fig. 1. Effect of heavy and light kernel damage upon test weight, semolina specks and macaroni color.

kernels upon macaroni color. The data are arranged in order of decreasing color score from left to right, and emphasize the conclusions already reached in respect to the influence of heavy kernel damage

upon this macaroni property. No degrading effect was noticeable with light damage until 25% concentration was reached, but as no blends were made between 10% and 25% some slight influence upon color may have been exerted at a lower proportion of damaged kernels. Twenty-five and 50% light, as well as 5% heavy damage, gave the same macaroni color score. Seventy-five per cent light damage markedly reduced the color while more than 5% of the heavy damage was extremely injurious.

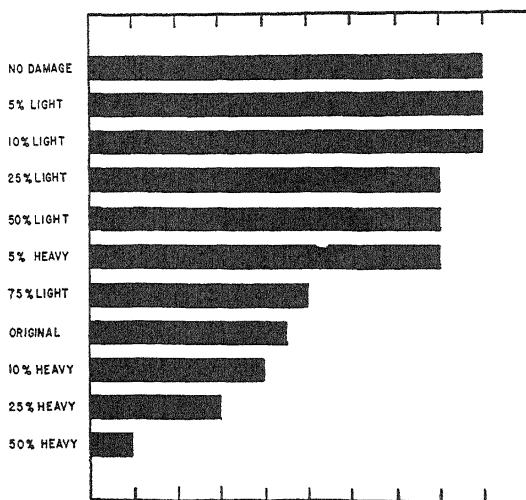


Fig. 2. Effects of different percentages of light and heavy kernel damage upon macaroni color score.

Summary and Conclusions

The belief of many chemists regarding the detrimental effects of different degrees of damaged durum wheat in the mill mix upon semolina and macaroni quality has been largely substantiated by the present investigation. Lightly damaged kernels showing discoloration at the tip without apparent injury to the other portions of the kernel can be tolerated in as high a proportion as 10% with good milling durum, while 25% does not greatly lower macaroni color or increase semolina speckiness. Higher proportions than 50% would be extremely hazardous to use in the mill mix.

The situation when heavily damaged kernels showing evidences of injury in the crease and other portions of the kernel are included in the blend is more critical. The presence of 5% of damaged grain signifi-

cantly affects the number of semolina specks and macaroni color score, while 10% is very detrimental.

In milling durum wheat damaged by "black point" and other infections special care is required in respect to the degree of damage of individual kernels permitted in the mill blend. If only light injury at the tip of the kernel is present the situation is not critical so far as proportion of damaged wheat is concerned, but a careful check should be kept upon the quality of the products while the blend is being milled. If heavy kernel damage is present, extreme diligence should be exercised to keep the quantity allowed to go to the mill at any time below 5%. It must also be remembered that if light as well as heavy damage is present, the effect upon quality will be additive and greater attention will have to be paid to the maximum quantity of heavily infected kernels allowed.

It was found that the grade was materially lowered by the presence of heavily damaged kernels, the addition of 50% resulting in decreasing the grade from No. 1 Hard Amber Durum to Sample Grade Durum. This would entail a serious financial loss to the grower. The presence of 5% of heavily injured grain lowered the grade to No. 3 Hard Amber Durum. Light damage was without appreciable effect upon the grade under the existing Federal grading regulations.

Acknowledgment

The authors wish to acknowledge assistance provided by the Work Projects Administration, Research and Records Division, through the operation of Seed Testing Project O.P. 165-1-73-144, during this investigation.

Literature Cited

- Binnington, D. S., and Geddes, W. F.
1936 Experimental durum milling and macaroni-making technique. *Cereal Chem.* 13: 497-521.
- Brentzel, W. E.
1941 Blight and other damage of durum wheat in 1940. (2) Effect on grain to be used for seed. *N. D. Agr. Exp. Sta. Bul.* 296.
- Fifield, C. C., Smith, G. S., and Hayes, J. F.
1937 Quality in durum wheats and a method for testing small samples. *Cereal Chem.* 14: 661-673.
- Harris, R. H., and Sibbitt, L. D.
1941 Blight and other damage of durum wheat in 1940. (1) Effect on macaroni-making quality. *N. D. Agr. Exp. Sta. Bul.* 296.
- 1942 Experimental durum milling and processing equipment with further quality studies on North Dakota durum wheats. *Cereal Chem.* 19: (previous paper, this issue).

THE INFLUENCE OF THE DRYING PROCEDURE ON MALT COMPOSITION¹

ALLAN D. DICKSON and H. L. SHANDS²

Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and University of Wisconsin, Madison, Wisconsin

(Received for publication November 3, 1941)

The influence of drying procedure on certain characteristics of finished malt has been known and used commercially for many years. Previous to 1900 Prior, as reported in Wahl and Henius's *Handy Book* (1902), studied this subject rather extensively with particular emphasis on the effect of drying temperature on some enzyme systems.

More recent literature agrees that drying reduces enzymatic activities, particularly diastatic power, but is contradictory on the effect of drying on color formation and the solubility of various nitrogen and carbohydrate constituents. The contradictions are not surprising since Kolbach and Schild (1935) showed that the effect of higher temperatures was largely dependent on the moisture content of the malt at the time high temperatures were applied.

In investigating the malting quality of different barley varieties, it was found that the drying procedure appreciably affected malt composition and was of importance in the evaluation of barleys. From 1935 to 1937, limited studies of drying procedures have been made at the Malt Laboratory at Madison. The results suggested an experiment where green malts produced under identical conditions were given drying treatments so planned that small changes in composition might be more easily interpreted.

Materials and Methods

The first study was made in 1939 on Wisconsin Barbless and Oderbrucker barleys grown in that year. Both were field samples, the Wisconsin Barbless from University Farm and the Oderbrucker from a farm near Madison. Quantities of the barleys were subdivided into 170-g samples (on a dry basis), and these were steeped to and malted at approximately 46% moisture for six days at 16°C (60.8°F). The malting was done in the small experimental chamber described by Shands, Dickson, and Dickson (1941). At the end of six days, one sample was used for green malt analysis, and the others were placed in

¹ Based on cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station. The United States Maltsters Association has cooperated through an industrial fellowship grant to the University of Wisconsin.

² Agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Assistant Professor of Agronomy, Wisconsin Agricultural Experiment Station.

the dryer. After they had received certain specified drying treatments, samples were removed, cleaned, and placed in friction top cans until analysis could be made. The drying treatment for each malt is shown in Table I. These drying conditions were designed to reduce moisture to a reasonably low level before the temperatures were increased. Samples of the malts were removed from the dryer at short intervals in order to follow changes in composition more closely.

The first series of malts was analyzed for moisture, diastatic power, extract, color, and the various nitrogen fractions of the laboratory worts. The green malts were frozen with dry ice and ground through a chilled food chopper. The ground malt was allowed to thaw and dry slightly before samples for moisture and diastatic power determinations were taken.

The methods of the American Society of Brewing Chemists (1937) were used, except for diastatic power, where the ferricyanide modification was used. The nitrogen fractions were determined according to the methods described by Dickson and Burkhart (1942).

The study was repeated the following year with two other samples of Oderbrucker and Wisconsin Barbless barley grown in farmers' fields in Wisconsin in 1940. Minor modifications in the drying temperatures and times were used as indicated in Table II. After a short preliminary kilning, two samples were dried further by phosphorous pentoxide at room temperature in a vacuum desiccator. These malts were analyzed in more detail, including total diastatic activity with papain extraction, alpha-amylase by the method of Blom and Bak (1938), and proteolytic power by a modification of the method of Kolbach and Simon (1936).

Drying Treatments in Kiln

The data on the first series, carried out in 1939, are given in Table I. In Table II are given the more detailed data obtained on the 1940 series of malts. The relation between moisture loss and the reduction of diastatic power during drying for the two varieties and the two years is shown graphically in Figure 1.

In three out of four malts, the diastatic value on the green malt was appreciably below that of a similar malt which had been dried at a relatively low temperature. The evidence indicated that this was caused by incomplete enzyme extraction from the green malt, rather than a further enzyme production during the early stages of drying. Drying was rapid enough to preclude any great increase in enzyme activation during the process, since the quantities of malt were small and the air velocity was high. Other work on the determination of diastatic power in green malt has indicated the difficulty of grinding

TABLE I
THE INFLUENCE OF TEMPERATURE AND TIME OF DRYING ON THE COMPOSITION OF MALTS
MADE FROM ODERBRUCKER AND WISCONSIN BARBLESS BARLEYS—1939 SERIES

Drying treatment ¹	Barley variety	Moisture %	Dia- static power °L	Ex- tract (dry basis) %	Color <i>Lap</i> 1/2	Wort nitro- gen %
None	Oderbrucker	47.1	207	—	—	—
2 hrs 45°C (113°F)	"	11.0	226	73.2	1.6	0.77
12 hrs 45°C (113°F)	"	6.1	221	72.9	1.9	0.78
12 hrs 45°C + 4 hrs 55°C (131°F)	"	5.6	218	72.7	1.9	0.76
12 hrs 45°C + 4 hrs 55°C + 4 hrs 65°C (149°F)	"	4.5	193	73.0	1.8	0.77
12 hrs 45°C + 4 hrs 55°C + 6 hrs 65°C	"	4.6	205	72.8	1.9	0.76
12 hrs 45°C + 4 hrs 55°C + 2 hrs 75°C (167°F)	"	3.7	165	72.6	1.9	0.77
12 hrs 45°C + 4 hrs 55°C + 6 hrs 65°C + 2 hrs 75°C (185°F)	"	3.2	135	72.3	2.0	0.77
2 hrs 45°C + 4 hrs 75°C	"	4.5	156	72.8	1.8	0.77
None	Oderbrucker	46.1	219	—	—	—
2 hrs 40°C (104°F)	"	10.7	217	72.9	1.5	0.74
26 hrs 40°C	"	6.3	221	73.1	1.6	0.75
50 hrs 40°C	"	6.1	230	73.2	1.6	0.77
50 hrs 40°C + 4 hrs 75°C	"	4.0	166	72.6	1.8	0.76
None	Wis. Barbless	45.6	118	—	—	—
2 hrs 45°C (113°F)	"	11.3	119	74.8	1.4	0.58
12 hrs 45°C	"	7.1	119	74.3	1.4	0.65
12 hrs 45°C + 4 hrs 55°C (131°F)	"	6.3	119	74.6	1.4	0.61
12 hrs 45°C + 4 hrs 55°C + 4 hrs 65°C (149°F)	"	4.9	107	74.6	1.4	0.61
12 hrs 45°C + 4 hrs 55°C + 6 hrs 65°C	"	4.7	97	74.6	1.4	0.60
12 hrs 45°C + 4 hrs 55°C + 2 hrs 75°C (167°F)	"	3.9	85	73.9	1.5	0.61
12 hrs 45°C + 4 hrs 55°C + 6 hrs 65°C + 2 hrs 75°C (185°F)	"	3.1	60	73.5	1.6	0.60
2 hrs 45°C + 4 hrs 75°C	"	4.3	85	74.0	1.5	0.59
None	Wis. Barbless	45.4	121	—	—	—
2 hrs 40°C (104°F)	"	11.0	118	74.7	1.4	0.62
26 hrs 40°C	"	6.9	121	74.3	1.4	0.59
50 hrs 40°C	"	6.3	112	74.3	1.4	0.61
50 hrs 40°C + 4 hrs 75°C	"	3.9	75	73.9	1.4	0.59

¹ All samples except green malts received 8 hrs drying at 25°C (77°F) plus 4 hrs at 35°C (95°F), plus additional treatment indicated.

TABLE II
THE INFLUENCE OF TEMPERATURE AND TIME OF DRYING ON THE COMPOSITION OF MALTS
MADE FROM ODERBRUCKER AND WISCONSIN BARBLESS BARLEYS—1940 SERIES

Drying treatment ¹				Barley variety	Moisture	Dia- static power	Dia- static power pain digestion	Alpha- amylase (Blom)	Proteoly- tic power (Kolbach) total mg of edestin N hyd	Extract dry basis	Color	Wort N as % of dry malt	Formol wort N as % of nitrogen	Maltose value of wort, % of wort solids
Hrs, 45°C, 113°F	Hrs, 55°C, 131°F	Hrs, 65°C, 149°F	Hrs, 75°C, 167°F	Hrs, 85°C, 185°F		%	%L			%	Lon 5.2	%	%	—
None					Oderbrucker	44.7	180	79	78	75.6	—	—	—	—
Green malt					"	10.5	204	75	96	75.2	1.3	0.71	13.9	69.0
12	4				"	7.1	234	82	55	75.5	1.1	0.71	16.9	68.9
12	4	4			"	6.1	251	82	54	75.5	1.3	0.71	18.0	69.1
12	4	4	4		"	4.9	238	79	55	75.3	1.3	0.72	17.5	68.3
12	4	6	2		"	4.4	228	81	52	75.2	1.3	0.71	17.6	69.8
12	4	6	2	2	"	4.2	226	80	55	75.2	1.3	0.71	18.0	69.4
12	4	6	2	2	"	3.3	162	81	49	75.1	1.3	0.71	18.0	68.5
12	4	6	2	4	"	2.8	155	81	57	75.0	1.5	0.71	18.7	67.7
2 + 43 days over P ₂ O ₅ —room temp.					"	2.7	148	79	52	—	—	—	—	—
None					Wis. Barbless	44.3	131	39	40	—	—	—	—	—
Green malt					"	10.7	146	43	36	74.2	1.0	0.55	16.8	69.0
12					"	7.1	214	47	39	74.1	1.0	0.56	17.8	69.0
12	4				"	6.1	210	47	39	73.8	1.0	0.55	18.1	68.8
12	4	4			"	4.9	215	45	35	73.9	1.1	0.57	16.3	68.2
12	4	6	2		"	4.6	183	46	28	73.5	1.3	0.55	17.3	68.8
12	4	6	2	2	"	4.3	175	49	26	73.6	1.3	0.55	17.3	68.7
12	4	6	2	2	"	3.5	164	42	33	73.7	1.3	0.56	16.4	67.8
12	4	6	2	4	"	3.1	111	42	37	73.6	1.4	0.55	17.1	67.6
2 + 43 days over P ₂ O ₅ —room temp.					"	2.9	142	43	37	—	—	—	—	—

¹ All samples except green malts received 8 hrs drying at 25°C (77°F) plus 4 hrs at 35°C (95°F), plus additional treatment indicated.

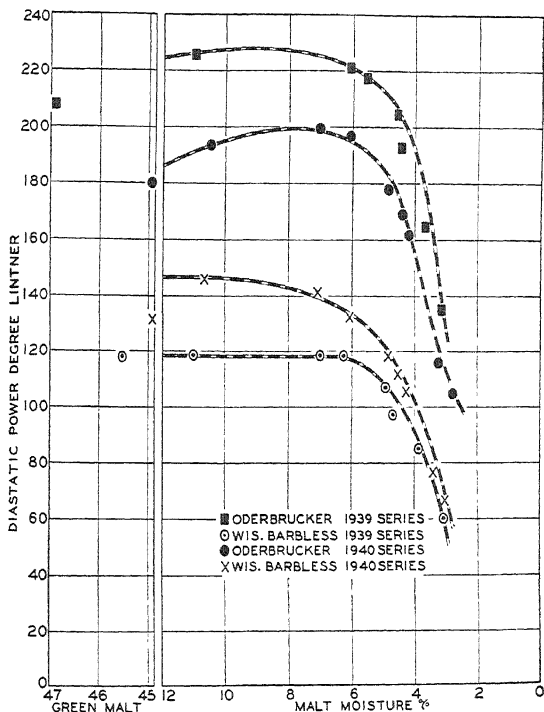


Fig. 1. The relation between moisture loss and the reduction of diastatic power during drying of malts made from Oderbrucker and Wisconsin Barbless barleys.

the sample sufficiently to obtain a complete extraction of the enzymes. The "total" diastatic power values obtained by using papain extraction are further evidence that the size of the particles in the ground material may have been too large to permit complete diffusion of the extraction medium.

At temperatures of 55°C (131°F) and below, when the moisture content of the malts was not reduced below 6%, there was no great reduction in diastatic power. With increasing high temperature and particularly below 5% moisture in the malt, the decrease in diastatic power was very rapid. There appeared to be a critical point in diastatic power when drying conditions reduced the moisture below 6%. In other studies of malting quality of different barley varieties made at the Madison Laboratory, malts have been dried to final moistures of between 5% and 6%. As indicated in this study, this procedure gives a more reliable measure of the potential ability of a variety to develop diastatic power, without introducing the variable of excessive kilning.

Long periods of drying at 40°C (104°F) produced practically no reduction in diastase but reduced the moisture only to approximately 6%. There is some evidence in the data of Table I that the enzymes were more stable to heat when the moisture content of the malt was low. Although the total reduction during drying was appreciably greater in the high-diastatic Oderbrucker malts, the percentage reduction was somewhat greater in the Wisconsin Barbless samples. Kopecky (1937) states that the richer the green malt in diastase, the greater is the loss of diastatic activity on the kiln. Apparently this is true for absolute values, but not when the two varieties, Oderbrucker and Wisconsin Barbless, are compared on a percentage basis.

The data given in Table II appear to substantiate the fact that alpha-amylase was much more stable to heat than beta-amylase, since there was practically no reduction in the former throughout the drying process. The values fluctuated within relatively narrow limits for both varieties and the variation was not consistently associated with drying treatment. It would seem that the reduction in total diastatic value caused by kilning treatments would be accounted for primarily by the inactivation of the beta-amylase component.

The data on proteolytic power, using a modification of Kolbach and Simons' (1936) edestin method, are difficult to interpret. Extraction of the enzymes from the green malt of the Oderbrucker samples appeared incomplete. A large reduction in activity was indicated between 2 and 12 hours' drying at 45°C, with little or no further decrease at the higher temperatures. The highest proteolytic power value on Wisconsin Barbless malts was obtained on the green malt, with a small decrease after a temperature of 65°C was reached. These limited data might indicate that the proteolytic enzyme systems of the varieties are different in their reaction to drying conditions. Heintz (1939) applied different kilning treatments to malts which showed a high germinative activity and found that proteolysis, as measured by the formol titration, required a temperature of 80°C for reduction and even then was not greatly altered by conditions. The results reported herein on Wisconsin Barbless coincide with the findings of Heintz. In other studies by Ayre and Anderson (1939) and unpublished data from this laboratory, a significant positive correlation has been obtained between proteolytic power and wort nitrogen. These data show that wort nitrogen was uninfluenced by the drying conditions. The absence of an apparent high correlation between two factors in the data from the Oderbrucker samples may be interpreted as evidence of the unreliability of the proteolytic power values. These discrepancies indicate that further study of the effect of drying conditions upon proteolytic

activity is desirable. More reliable methods for determining proteolytic activity need to be developed.

Extract content of the malts did not seem to be greatly affected until temperatures of 75°C to 85°C (167° to 185°F) were reached, when a small reduction was noted. The magnitude of the reduction varied from 0.5% to 1.3% when moisture was reduced from about 10% to 4% or less. The average loss was 0.8%. Maltose values of the wort showed reductions about twice as large as those for extract. Under the drying conditions used, no great increase in color took place, but there was a slight increase at a temperature of 85°C.

Total wort nitrogen appeared to be uninfluenced by drying conditions. In the Oderbrucker samples, formol nitrogen increased very slightly and fairly regularly with increased drying. This fact is of doubtful significance, since a similar trend was not apparent in the Wisconsin Barbless samples. There was an indication that at the higher drying temperatures slightly more of the wort nitrogen became permanently soluble. Kolbach and Schild (1935) showed that the changes in the nitrogen constituents were greatly dependent upon the moisture content of malt and kilning temperature. They found that at high moisture and high temperatures the increase in permanently soluble and formol nitrogen was appreciable. In the present study, the moisture of the malt was reduced to approximately 10% before high temperatures were applied. The results agree more closely with those of Trkan and Zila (1937), who applied different kilning treatments to malts containing 9.0% moisture and found that neither time of curing nor finishing temperatures altered the proportion of the individual nitrogen fractions.

The results reported herein point to a probable explanation of the low diastatic values obtained by Dickson *et al.* (1935) for the 1934 malts. It would appear that less severe kilning conditions would be more appropriate in comparing the ability of different barley varieties to develop enzymatic systems during malting.

Obviously the above data apply only to the drying conditions outlined. However, it is felt that the information should be applicable to commercial kilning procedures used in this country in the production of pale malts. The effective air velocity in commercial kilns is probably less than that used here with small quantities of malt. In most commercial houses the moisture of the malt is about 10% when dropped to the lower kiln for high-temperature drying. The results reported should apply to changes taking place during the final finishing of this malt and could be used to modify drying procedures in commercial houses.

Treatments Other Than Kilning

During the drying of malt, both temperature and changes associated with the loss of moisture may be active in reducing enzymatic activity. It was desired to dry malts to a moisture content of about 3% without the use of high temperatures. As noted in Table I, after 50 hours' drying at 40°C the moisture was reduced only to approximately 6%. The diastatic power was reduced very little if at all below the value for green malt. In order to reduce the moisture without the use of heat, a sample of malt from each variety, containing approximately 10% moisture, was dried for 43 days in a vacuum desiccator over calcium chloride and phosphorous pentoxide. The results are given in Table II. The moisture was reduced to less than 3%, but the diastatic-power values were reduced only from 193 to 176 for Oderbrucker and from 146 to 122 for Wisconsin Barbless, or 9% and 16%, respectively, for the two varieties. With these two samples it appears that drying over phosphorous pentoxide produces a proportionately greater reduction of the inactive diastase, liberated by papain, than of the free form. Verification of this would require further study. In some earlier studies of drying at room temperature, five high-diastatic malts containing from 6% to 8% moisture were dried 43 days over phosphorous pentoxide. The percentage reduction of diastatic power varied from 8% to 20% and the final moistures were from 2.5% to 3.0%.

Replicated samples of the same malts were submitted to heat treatments for 6 and 12 hours at 80°C (176°F) in closed metal cans in order to limit the loss of moisture as much as possible. The results were very erratic and the variation between replicates large, but the percentage reduction in diastatic power varied from 40% to 85%. If a comparison of these two treatments can be justified, the data indicate that high temperature is much more important in the reduction of diastatic power than the loss of moisture.

Summary

A series of malts produced from Oderbrucker and Wisconsin Barbless barleys under uniform conditions were submitted to a drying schedule involving increasing temperatures from 25° to 85°C. Samples were removed at intervals in order to study the effect of time and temperature of drying on malt composition. Above 55°C and at moisture contents below 6%, the reduction in diastatic power was rapid. Alpha-amylase was reduced only slightly if at all. Extract, color, and maltose values of the wort were influenced slightly at the higher temperatures. The effect of the drying procedure on the nitrogen fractions of the wort was very slight.

Acknowledgments

The authors acknowledge the assistance of B. A. Burkhart, L. A. Hunt, and W. W. Pregler in preparing and analyzing the malts herein reported. Assistance in the preparations of these materials was furnished by the personnel of the Works Project Administration Project No. 65-1-53-2349.

Literature Cited

- American Society of Brewing Chemists
1937 Official methods of analysis. Chicago, Ill.
- Ayre, C. A., and Anderson, J. A.
1939 Varietal differences in barleys and malts. VI. Autolytic proteolytic activity of malt and its correlations with wort nitrogen and barley nitrogen fractions. *Can. J. Research* **C17**: 239-246.
- Blom, J., and Bak, A.
1938 Über die Bestimmung von Amylasen durch Verflüssigung von Stärkekleister. *Hoppe-Seyler Z. physiol. Chemie* **256**: 197-207.
- Dickson, J. G., Shands, H. L., Dickson, A. D., and Burkhart, B. A.
1935 Barley and malt studies. I. Developing new varieties of barley for malting and their properties. *Cereal Chem.* **12**: 596-609.
- Dickson, A. D., and Burkhart, B. A.
1942 Changes in the barley kernel during malting: chemical comparisons of germ and distal portions. *Cereal Chem.* **19**: 251-262.
- Heintz, L.
1939 Determination of germinative, diastatic, and proteolytic powers as factors for evaluation of malt. *Schweitzer-Brau. Rundschau* **50**: 95-109. *Cf.* *J. Inst. Brewing* **45**: 351.
- Kolbach, P., and Schild, E.
1935 Influence of moisture and temperature upon the change of substances during kilning of malt. *Wochschr. Brau.* **52**: 129-134, 137-141, 147-150.
- Kolbach, P., and Simon, H.
1936 Über die proteolytische Kraft von Malzanszügen und Würzen. *Wochschr. Brau.* **53**: 297.
- Kopecky, O.
1937 Change in diastatic power of green malts in the course of kilning. *Petit J. brasseur* **45**: 78-79. *Cf.* *J. Inst. Brewing* **43**: 119.
- Shands, H. L., Dickson, A. D., and Dickson, J. G., and B. A. Burkhart.
1941 The influence of temperature, moisture, and growth time on the malting quality of four barley varieties. *Cereal Chemistry* **18**: 370-394.
- Trkan, M., and Zila, U.
1937 *Allgem. Brauer- u. Hopfen-Z.* **77**: 266. *Cf.* *Int. Brew. Abstracts* **5** (1937): 543-580.
- Wahl, R., and Henius, Max
1902 *Handy Book of brewing and malting* (2nd ed.). Chicago, Ill.

A SIMPLE METHOD FOR THE APPROXIMATE ESTIMATION OF PROTEOLYTIC ACTIVITY

QUICK LANDIS

The Fleischmann Laboratories, Standard Brands Incorporated, New York

(Read at the Annual Meeting, May 1941)

In 1894 Mett¹ allowed gastric juice to act on coagulated egg albumin contained in small open-ended glass tubes and observed that the coagulum was digested from the open end. The rate of digestion was thus a rough measure of the concentration of the enzyme in the gastric juice, provided the rate of diffusion did not become a limiting factor.

¹ Mett, *Arch. Anat. Physiol.*, p. 68, Verda (1894).

This simple method has been applied to the determination of proteinases of the papain type, using gelatin as a substrate. After some preliminary experiments the apparatus shown in Figure 3 was developed. A set of tubes are filled by inverting in a small rack and pipetting the substrate solution at 30°C into the open end of each tube. They are then covered with a beaker to minimize evaporation and placed in the ice box overnight.

From 20 to 40 ml of the enzyme infusion is placed in a 50-ml flask, a prepared tube inserted, and the assembly placed in a thermostat at

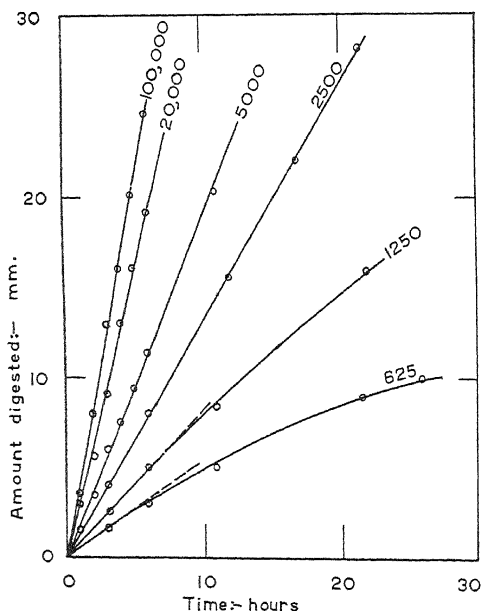


Fig. 1. Rate curves. The concentration of enzyme (ficin) in milliunits per gram (arbitrary scale) is given on each curve

20°C. After a convenient interval the tube is removed, the digested fluid gently shaken from the end, and the length of material which has been digested is measured. A typical set of curves is given in Figure 1. Under these conditions the amount of digestion is directly proportional to the time over a considerable range.

This method also facilitates the study of activators; since no specified volume of enzyme infusion is required the results are obtained directly in terms of concentration of active enzyme in the infusion. A series of stock solutions can conveniently be used.

The "regular" stock solution which has been found satisfactory is a 0.5M solution of NaCl in an acetate buffer solution of 5.0 pH which is 0.5M in acetate. Boiled, distilled water is used in making the solutions. Various "activator" stock solutions, with the same buffer and NaCl concentration but 0.05 to 0.3N in cyanide, have been used, with suitable precautions to maintain the final NaCl, acetate, and H-ion concentration unchanged. These solutions are diluted 1:10 in preparing either the substrate for the Mett tubes or the enzyme infusions.

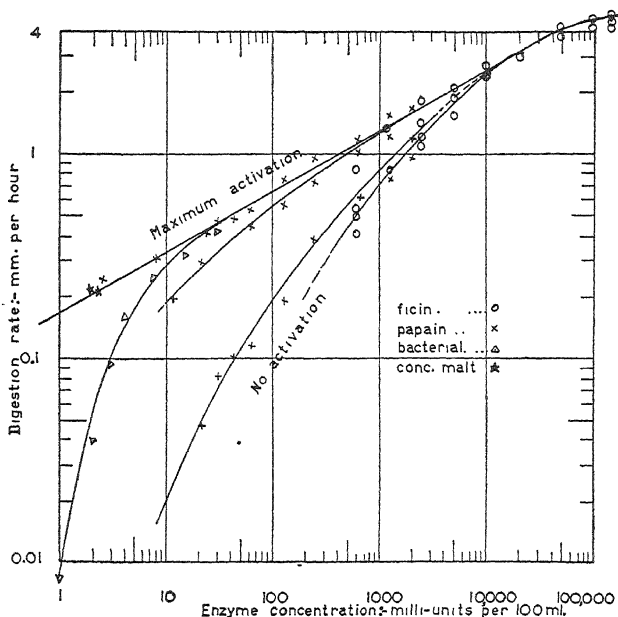


Fig. 2. Concentration relationships. Maximum activation was obtained with the special activator stock solution described in the text.

A concentration of 1.5% gelatin has been found satisfactory for the substrate.

A special activator stock solution was used to obtain the maximum activation as will be shown later. This was prepared from one of the solutions previously described which was 0.3N in cyanide. To 500 ml of this solution was added 50 g of ground malt. The mixture was saturated with H_2S and after one hour's gentle maceration the supernatant liquor was centrifuged off and boiled to expel excess H_2S and destroy the malt enzymes. This makes available the natural activators in their reduced form for subsequent experiments.

Some results obtained by this method are indicated in Figure 2. It will be observed that as the enzyme concentrates are diluted their activity diminishes disproportionately. Whether this is due to the presence of inhibitors in the water used for dilution, to an inadequate supply of protective substances in the original material, or to an inherent characteristic of the enzyme, is not clear. However, by the use of maximum activation some enzymes at great dilution can be made to coincide in their activity with that deduced from the more

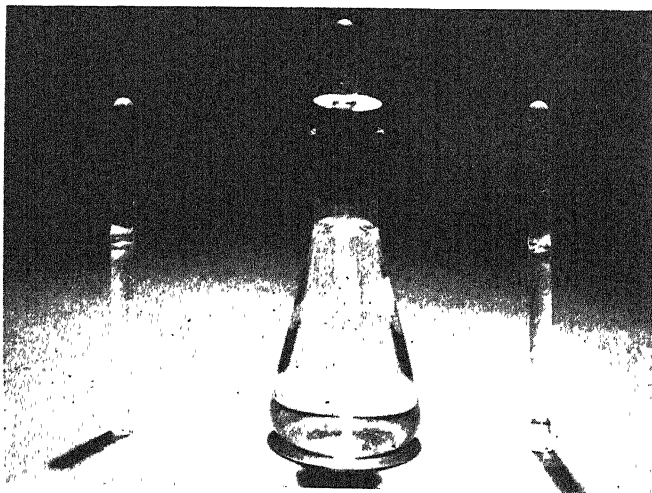


Fig. 3. Apparatus. Glass tubes (6 mm. id) are sealed to glass rods of the same diameter forming a tube closed for half of its length. The gelatin is prevented from melting in the lower end of the tube by the water bath.

concentrated infusions. The common activators used, however, had no effect on a bacterial protease, although it is possible that its dilution function is different from that of the papain-type enzymes.

Summary

A modified Mett-tube procedure using gelatin for the determination of proteinase concentration is described and an example of its application to the study of activation is given.

HYSTERESIS OF AIR-DRY WHEAT STARCH¹

HENRY C. REITZ, ROSS AIKEN GORTNER, and RAYMOND E. CARLSON

Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota

(Received for publication October 14, 1941)

It is well recognized that colloid gels undergo hysteresis or aging with time and that starch pastes show such hysteresis effects to a marked degree.² However, we were surprised to find an apparent hysteresis effect taking place in air-dry starch (moisture 10.57%), especially in view of the recommendation of Ripperton (1931) that the viscosity values of different starches should be expressed in terms of a standard starch instead of in absolute viscosity units. Ripperton (1931, p. 154) states that "air-dry starch is very stable in its properties and makes a dependable standard over a period of years." It is the purpose of this note to call this statement in question.

Experimental

A large sample of wheat starch was isolated from *Triticum vulgare* var. Thatcher by centrifuging techniques, the technique being such as to remove all small or broken granules and to yield a pure white air-dry starch containing remarkably uniform granule sizes. The starch as bottled contained 10.57% moisture, 0.041% nitrogen, and 0.180% ash. The ash was almost wholly derived from the phosphoric acid content (0.1588% P_2O_5 in the dry starch). During our experiments the starch has been kept in a sealed glass container.

Viscosity studies were made on the sample by the cold gelatinization technique of Ostwald and Frankel (1927), using NaOH as the gelatinizing reagent. The gelatinization and viscosity-determination procedures were rigidly standardized and all experimental details exactly followed during each series of runs. For the purpose of this note it is not essential to describe these procedures in detail.² Suffice it to say that the starches were gelatinized by the appropriate concentration of NaOH at 30°C for a two-hour period. The flasks containing the starch and NaOH solution were slowly turned in a mechanical rotator immersed in the constant-temperature bath during the gelatinization period. At the end of the period the viscosity was determined at $30^\circ \pm 0.03^\circ\text{C}$, using an Ostwald viscometer made of pyrex glass, having a volume of 19.54 ml, a capillary length of 9.76 cm, and a capillary diameter of 0.188 cm. The viscometer employed was carefully standardized against glycerol solutions of known viscosity. The viscosities were calculated in centistokes.

¹ Paper No. 1931, Scientific Journal Series, Minnesota Agricultural Experiment Station.

² The techniques are detailed in the manuscript copy of a Ph.D. thesis by H. C. Reitz entitled "A Comparative Study of the Starches of the *Triticum* Species," University of Minnesota, June, 1938. On file in the Library of the University of Minnesota.

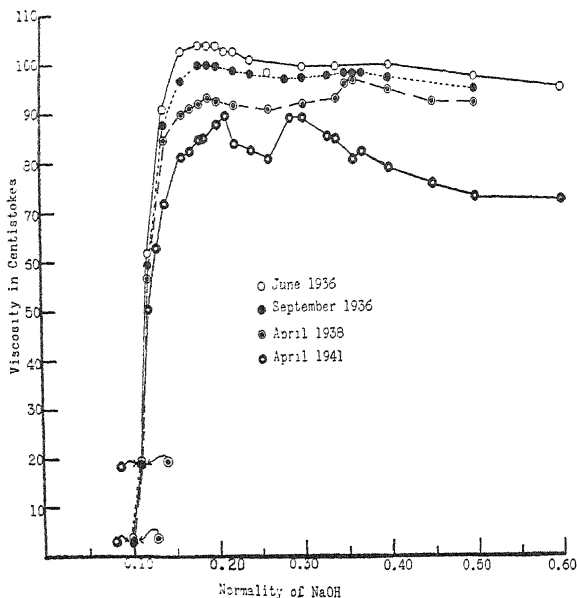


Fig. 1. Showing hysteresis in the viscometric behavior of a 2% "Thatcher" wheat starch sol cold gelatinized with various concentrations of NaOH at 30°C.

Four series of runs at different times are shown in Figure 1. It is evident from these curves that the starch sample has changed progressively with time, aging resulting in a marked lowering of the maximum viscosity which is attained.

At present we are not theorizing on the form of the viscosity curves, but apparently aging results in a shifting of both the maxima, which originally occurred at approximately 0.18*N* and 0.35*N* NaOH. Aging apparently shifts the first maximum to a higher NaOH concentration and the second maximum to a lower NaOH concentration, and greatly accentuates the dip between the maxima.

Summary

Air-dry wheat starch undergoes hysteresis with time as evidenced by cold gelatinization viscosity behavior. An air-dry wheat starch cannot be used over a period of years as a viscometric standard.

Literature Cited

- Ostwald, W., and Frankel, G.
 1927 Kinetische Studien über Stärke-Verkleisterung. I. Über Kaltverkleisterung von Stärken. *Kolloid Z.* 43: 296-312.
 Ripperton, E. T.
 1931 Measurement of consistency of starch solutions. *Ind. Eng. Chem. (Anal. Ed.)* 3: 152-154.

CEREAL CHEMISTRY

VOL. XIX

JULY, 1942

No. 4

INTERPRETATION OF VISCOSITY MEASUREMENTS ON STARCH PASTES¹

B. BRIMHALL and R. M. HIXON

Iowa Agricultural Experiment Station, Ames, Iowa

(Received for publication March 10, 1942)

The publications on anomalous viscosity² and gel properties have been accumulated by technologists working on diversified colloidal products such as tar, paint, plastics, proteins, starches, etc. They are scattered through the various scientific and applied journals and available only in limited scope to the technologists in any one field. The use of particular instruments and the expression of results in specialized form in the different industries is generally a question of habit or convenience rather than of necessity.

A study of this diversified literature was made in search of methods best suited for characterizing starch, the study being a part of the corn breeding program looking forward to the variations in starch properties possible with modern hybridization techniques. This paper attempts to emphasize the connecting thread which runs through this literature and make a knowledge of it available to those interested in its application to starch.

Measurement of Viscosity

The different viscometers and their operation have been adequately described by Bingham (1922), Nivling (1924), Hatschek (1928), Barr (1931), Samec and Blinc (1937), and others. Only two general types are used to any extent for starch pastes:

1. *The capillary type:* This has found widespread use in the laboratory for theoretical studies because it is inexpensive and adaptable. The Ostwald pipette is a familiar example. For routine work on starch it often takes the form of a funnel or a metal cup with a small hole in the bottom, such as the Scott viscometer. Viscosity can be calculated from the rate of flow of the liquid through the capillary or orifice. Ordinarily viscosity is not calculated, but the pastes are com-

¹ Journal Paper No. J-986 of the Iowa Agricultural Experiment Station, Project 426. Supported in part by a grant from the Corn Industries Research Foundation.

² An explanation of the various rheological terms is clearly presented by Bingham (1930).

pared on the basis of time required for the flow of a certain volume of liquid.

2. *The rotational type:* Measurements depend on the torque produced on a suspended disk or cylinder as the cup containing the liquid is rotated around it. In some cases the disk is rotated in the stationary liquid. The MacMichael and Stormer viscometers belong to this type. The consistometer (Caesar, 1932; Caesar and Moore, 1935; Radley, 1940) and amylograph are instruments based on the same principle as the rotational viscometer. They are designed for use with very concentrated pastes and give results in which structural viscosity is subordinated to the resistance of the granules to crushing and the thixotropic characteristics of the pastes.

Figure 1 is a diagram of the apparatus used to obtain data for this paper: The pyrex test tube *A* (4.4 cm diameter, 20 cm deep), which

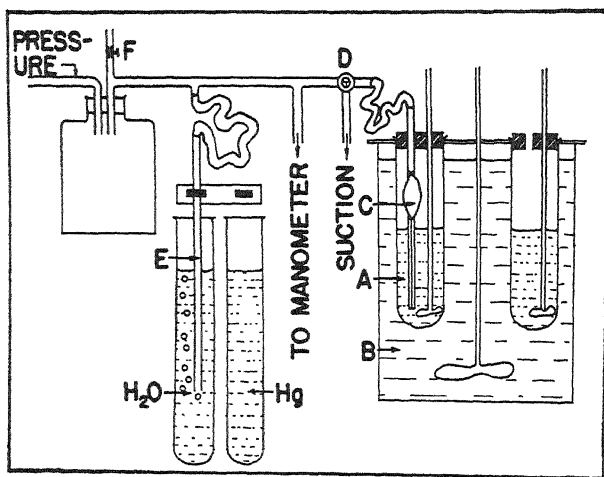


Fig. 1. Apparatus for measuring viscosity.

holds the paste, is immersed in a water bath *B*, the temperature of which is controlled by a thermoregulator. The glass water bath, provided with stirrer and heating coil, is large enough to hold six tubes of paste. The capillary pipette *C* is connected to a three-way stopcock *D* so that suction may be applied to draw the paste up into the capillary or air pressure applied to force the paste down again.

The pressure may be varied by raising or lowering tube *E* in the column of water or mercury surrounding it. A steady stream of bubbles from tube *E* is assured by allowing excess pressure to escape

at F . In this way, pressures up to 300 cm of water can be obtained quickly and accurately. However, it is best to avoid such high rates of shear when possible by using lower concentrations of starch or larger capillaries. The temperature is variable within the range from 25° to 100°C. Measurements are not made above 90° because suction on the capillary causes the paste to become filled with bubbles.

After the water bath has reached the desired temperature, the cold starch-water suspension is poured through the funnel into the viscometer tube, in which a mechanical glass stirrer is rotating at a speed of about 500 rpm. A viscosity-time curve should be obtained for each type of paste in order to find if and when it approaches a relatively constant value for measuring. This enables one to avoid the sharp initial rise and fall from the maximum often observed during the first few minutes of heating (Richardson and Waite, 1933; Briggs and McCarthy, 1942; Katz, Desai, and Seiberlich, 1938). In the apparatus shown in Figure 1, 40 minutes of heating at 90° is sufficient for unmodified cornstarch pastes, although it will vary with concentration of paste, type of container, and the like. After heating the required time, stirring is stopped, the clean capillary introduced, and time of flow determined at various pressures. Viscosity in poises is calculated by Poiseuille's law:

$$\eta = \frac{\pi R^4 g}{8LV} PT \quad (1)$$

where P = pressure (cm H₂O); T = time of flow (sec); R = radius of capillary; L = length; V = volume; and g = acceleration of gravity.

Eight capillaries ranging from 0.035 to 0.075 cm radius made it possible to measure pastes containing as high as 6% by weight of starch at 90° and higher concentrations at lower temperatures. Experiments using capillaries of six different sizes on one starch paste showed that they can be used interchangeably without appreciable error provided the time of flow is kept in the range between 30 and 200 seconds and there is not too large a spread in capillary dimensions.

The viscosity apparatus described above is similar to that of Richardson and Waite (1933). The reader is referred to their manuscript for an account of the factors affecting viscosity as well as the procedure for preparing and measuring starch pastes. The curves in Figure 2 are typical of the variation in viscosity of a starch paste with rate of shear. Many different theories have been advanced to explain this phenomenon, which is characteristic of systems showing structural viscosity. The experiments of McDowell and Usher (1931) clearly show that the peculiar physical properties of such systems are due to

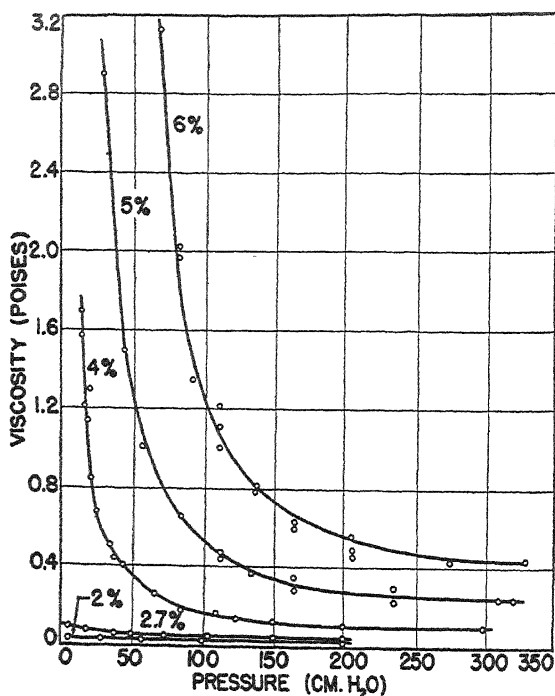


Fig. 2. Viscosity of unmodified cornstarch pastes at 90°C at different pressures and concentrations.

aggregates of particles which surround portions of the solvent and thus increase the effective concentration of solid material. Higher rates of shear tend to disorganize these aggregates to give a more uniformly distributed system. Under the conditions shown in Figure 2, starch suspensions of concentrations below 2% behave as true liquids.

Results on unmodified cornstarch pastes prepared at 90° are plotted in Figure 2 (viscosity-pressure) and Figure 3 (viscosity-concentration). The data at 70° and 80° give similar curves except that structural flow sets in at higher concentrations (7% at 70°; 5% at 80°; 2.7% at 90°). All measurements were carried out on the same batch of starch from one company, which uses somewhat larger amounts of SO₂ in its milling operation than most of the other wet-milling companies.

The following considerations may be applied in general to data from rotation-type viscometers as well as capillary types if it is remembered that rate of rotation corresponds to pressure applied. The

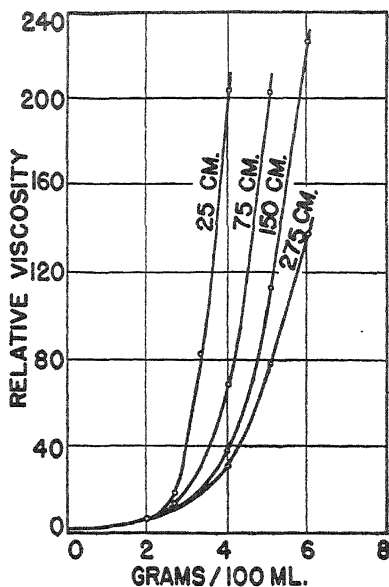


Fig. 3. Viscosity-concentration relationships for unmodified cornstarch pastes at 90°C at different pressures (cm H₂O).

discussion is divided into two parts, one dealing with flow-pressure relationships and the other with viscosity-concentration relationships.

Flow-Pressure Relationships

When rate of flow (V/T) is plotted against pressure, curves like those in Figure 4 are obtained. A straight line proceeding directly from the zero point is characteristic of true liquids such as water; with anomalous liquids like starch pastes, the curves follow the pressure axis for a time before rising and straightening out. Bingham (1922) suggests extrapolating this straight portion back to zero rate of flow (dotted line, Fig. 4) to obtain a "yield shear value," f . Hypothetically this amount of pressure must be applied in overcoming plastic or elastic forces before the paste begins to flow. There have been serious objections to the characterization of starch pastes by yield shear value: (1) Flow actually takes place below the yield value; thus it does not agree with Bingham's definition and has no physical meaning (Farrow, Lowe, and Neale, 1928; Hatschek, 1928). (2) Attempts to characterize modified starches by this method have failed because of the gradual slope of the curves obtained (Gallay, 1936; Porst and Moskowitz,

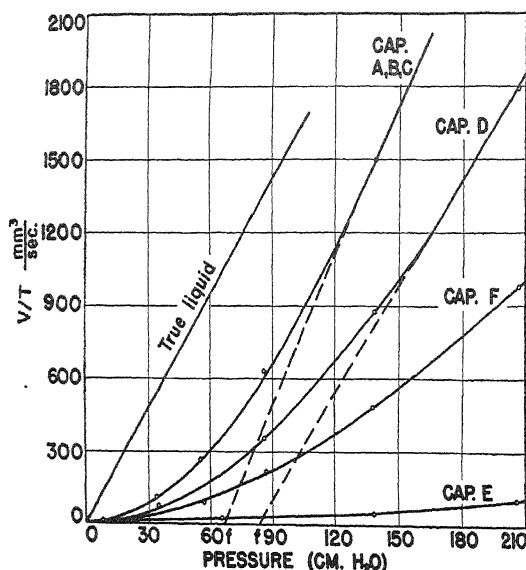


Fig. 4. Variation in rate of flow with pressure for a 4% cornstarch paste at 90°C using different capillary sizes.

1922). Figure 4 shows that extrapolation is entirely too arbitrary in nature, depending upon capillary size as well as the paste.

A slightly different treatment, based on the concept of plug flow, is subject to the same criticism. In this method, the slope of the horizontal portion of the curve, attributed to plug flow through the capillary, is assumed to be in constant ratio to the slope of the vertical portion where plug flow has been replaced by true liquid flow. This ratio has been used to characterize clay pastes (Scott Blair and Crowther, 1929) and flours (St. John, 1927).

A significant advance resulted from the use of logarithmic functions to make straight lines of the troublesome flow-pressure curves. If the logarithm of pressure is plotted against the logarithm of rate of flow (V/T), there are obtained straight lines whose slope is the same for any one paste (within certain limits) regardless of the dimensions of the capillary or type of viscometer. However, the lines will be displaced on the pressure axis according to capillary dimensions. This difficulty was surmounted (Farrow, Lowe, and Neale, 1928; Rabinowitsch, 1929) by splitting the viscosity equation into two parts representing the stress applied to the paste ($RPg/2L$) and the rate of shear

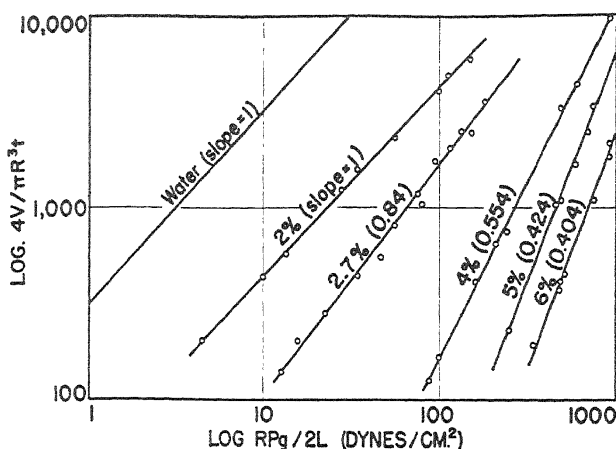


Fig. 5 Shear-stress curves for unmodified cornstarch pastes at 90°C at different concentrations.

produced ($4V/\pi R^3T$). When logarithms of these values are plotted against each other, they give a line whose position is independent of capillary size, its intercept as well as its slope being characteristic of the paste. Rabinowitsch (1929) presents a mathematical justification for this procedure.

When calculated in terms of shear and stress, results from rotation and capillary viscometers may be correlated and expressed by the same constants (Farrow, Lowe, and Neale, 1928). Figure 5 represents shear-stress curves for cornstarch pastes at different concentrations. They fit the equation:

$$\log 4V/\pi R^3T = M \log RPg/2L + \log C \quad (2)$$

where M is the slope of the line and C the intercept. While the meaning of these constants is not thoroughly understood, it appears that the slope M expresses the degree of structural flow of the paste. For water and a 2% paste, which do not show anomalous viscosity, $M = 1$. Likewise, the intercept C probably is a measure of the comparative amount of stress which must be applied to produce a given rate of shear in the several pastes; the value of C becomes larger as the viscosity of the paste increases.

Table I shows data of this type for a number of different unmodified starches. There is a constant relationship between slope and intercept for any one starch at different concentrations after structural flow has set in, as shown by the relatively constant value of $(I \times S)$ in the last

column of the table. Since slope and intercept are so closely bound up with one another, it seems improbable that each can be definitely associated with a distinct physical characteristic of the paste.

TABLE I
INTERCEPT-SLOPE RELATIONSHIP FOR STARCH PASTES

Starch	Concentration %	Intercept ¹ <i>I</i>	Slope ¹ <i>S</i>	Intercept \times slope ² (<i>I</i> \times <i>S</i>)
Commercial I.....	0	-2.42	1.00	0.58
(heated and measured at 90° C)	2.0	-1.62	1.00	1.38
	2.7	-0.98	0.84	1.70
	4.0	+0.76	0.554	2.08
	5.0	+1.40	0.424	1.86
	6.0	+1.64	0.404	1.87
Reid Yellow				
Dent cornstarch.	2.15	-1.62	1.00	1.38
(heated and measured at 90°)	2.6	-0.23	0.700	1.94
	3.0	+0.35	0.577	1.93
	4.0	+1.20	0.424	1.78
	5.0	+1.80	0.296	1.42

STARCHES, 2.7% CONCENTRATION, HEATED AT 99°, MEASURED AT 25° C

Reid Yellow Dent.....	+0.71	0.510	1.86
Commercial Corn I.....	0.53	0.554	1.95
Commercial Corn II.....	0.40	0.625	2.12
Potato.....	1.50	0.554	2.50
Tapioca.....	0.95	0.577	2.28
Rice.....	0.40	0.601	2.04
Waxy Maize.....	0.62	0.625	2.26
Popcorn.....	0.30	0.662	2.18
Mandan White Flour.....	0.56	0.601	2.04
Country Gentleman.....	-0.27	0.753	2.06

¹ These refer to intercept on the $RPg/2L$ axis and slope on the $4V/\pi r^3 T$ axis.

² A value of +3.00 was added to all intercept values to make them positive before multiplying.

Viscosity may be calculated from the curves in Figure 5 by the equation:

$$\log \eta = \log RPg/2L - \log 4V/\pi R^3 T \quad (3)$$

since viscosity is equal to the reciprocal of the tangent at any one point.

A set of curves plotted in a similar manner is shown by Galloway and Bell (1936), who obtained data on acid-modified starches using a MacMichael viscometer. The curves were linear within the limits of shear employed, the slope decreasing with increasing modification of the starch. When measurements were carried out over a wide range of shear values, transitions from one type of flow to another (which they designate as structural, laminar, and turbulent) were detected by changes in direction of the logarithmic flow-stress curves. These authors applied the equation:

$$F = KP^n \quad (4)$$

previously used by Farrow and Lowe (1923), where F = rate of flow, P = pressure, and n is the slope of the line corresponding to M in equation (2). The intercept K , however, contains in it the instrument constants and can be used to characterize the starch only when the same instrument is used in all cases.

Philippoff (1935, 1936) considers that the straight line of the log shear-log stress graph is merely part of an S-shaped curve which has a slope equal to that of water at high rates of shear and curves inward at low rates of shear where proportionately more stress is required. Farrow, Lowe, and Neale (1928) experimentally demonstrated the latter assumption, believing that the elastic properties of the paste become more prominent than its viscous flow at very low rates of shear. This observation is important because it emphasizes the desirability of using gel strength or elasticity measurements to characterize the starch at regions where viscosity determinations fail.

Viscosity-Concentration Relationships

The Einstein equation provided the theoretical foundation for viscosity equations which express concentration in terms of volume occupied by the solid phase. For a colloidal suspension of undeformable spheres he proposed the equation

$$\eta_r = 1 + K\phi \quad (5)$$

where η_r = relative viscosity and ϕ is the volume of spheres per unit volume of suspension. In other words, viscosity is independent of the size of the individual spheres but is a linear function of their total vol-

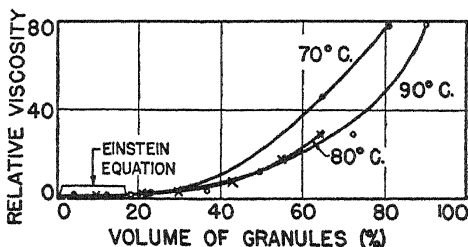


Fig. 6. Relationship between the volume of swollen granules and relative limit viscosity for cornstarch pastes at different temperatures.

ume. The equation is applicable only to the region of the viscosity- ϕ curve where the volume of spheres is relatively small and the curve is linear (Fig. 6). The constant K , representing the slope of the straight-line portion, has been the subject of considerable dispute. Einstein originally evaluated it at 2.5, while Hatschek (1913) believed that 4.5

was more nearly correct. In a specific application to starch, Harrison (1911) found that 4.75 better suited his experimental results. In an attempt to apply Harrison's equation, values of relative viscosity and volume of centrifuged granules found experimentally in this laboratory were used to calculate K . Rather than being constant, it varied all the way from 2 to 6 for unmodified cornstarch pastes. This equation has been discussed further by Humphrey and Hatschek (1916), working with unpasted suspensions of rice starch in carbon tetrachloride, and by Gally (1936) for acid-modified potato and cornstarch pastes.

For practical purposes this type of equation is not suited for characterizing starch pastes since: (1) It applies only to low concentrations where structural viscosity is lacking. The measurement which is generally desired is the degree of structural viscosity at higher concen-

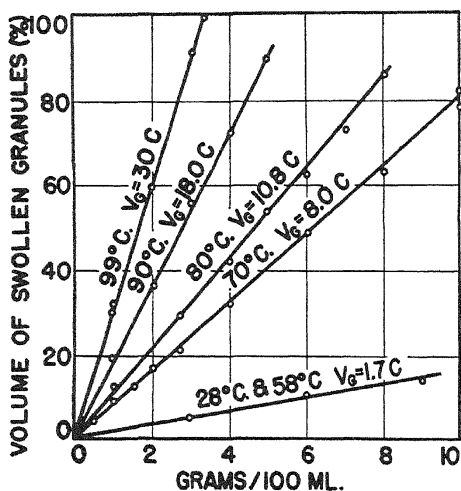


Fig. 7. Relationship between volume of swollen granules (V_g) and concentration of starch (C) at different temperatures.

trations; (2) ordinarily only the weight and not the volume of particles is known accurately (Hatschek, 1928). Starch granules in paste form are seldom undeformable or perfect spheres as the equation demands. This, as well as lack of smoothness and difference in the size of adsorption envelopes on the granules, has been suggested (Hatschek, 1913) to account for the variation in the value of K .

A linear relationship was found between the concentration of starch and the apparent volume of granules at any one temperature of cooking (Fig. 7). The pastes were prepared as described above for viscosity determinations, cooled in running water to room temperature, then

centrifuged in graduated tubes for $\frac{1}{2}$ hour at 1,750 rpm (15 cm radius). The volume of granules is largely a consequence of the temperature used to prepare the paste; thus by using volume rather than weight of starch in calculations, results at different temperatures may be correlated. In Figure 6, relative viscosities at 70°, 80°, and 90°C have been plotted against the apparent volume of granules (V_g) for an unmodified cornstarch. The difference between the curves at 70° and 90° is probably due to a change in the physical state of the granules, such as deformability and tendency to pack in the centrifuge tubes.

Table II summarizes the relative volumes of granules at different temperatures for five starches. The value (V_g/c) is the volume in milliliters occupied by 1 gram of starch in 100 ml of paste after heating at a given temperature and centrifuging.

TABLE II
VOLUME OF CENTRIFUGED GRANULES FOR STARCH PASTES SWOLLEN
AT VARIOUS TEMPERATURES

Temp.	V_g/c (%)				
	Wheat	Corn	Rice	Tapioca	Potato
°C	%	%	%	%	%
28	1.6	1.73	1.8	1.73	1.6
49	1.7	1.73	1.86	1.73	1.6
63	3.15	2.38	2.22	15.3	24.0
70	7.0	8.0	5.3	32.0	34.2
80	8.7	10.8	12.0	41.5	42.5
90	12.6	18.0	24.0	33.5	53.2
99	13.3	30.1	28.6	15.3	66.5

The tendency for tapioca granules to become ruptured above 80° is reflected in viscosity and rigidity of tapioca as well as in the decreased volume of granules. This decrease becomes less pronounced if the rate of stirring is lowered during preparation of the pastes, as would be expected.

Another equation involving the volume of granules is that of Hatschek (1913). He believes that at sufficiently high rates of shear, the viscosity becomes constant because the elastic particles become deformed and remain so throughout the flow period, provided they occupy at least 50% of the volume. Under these conditions he proposed the equation:

$$\eta_r = \frac{1}{1 - \sqrt[3]{\phi}} \quad (6)$$

to express relative viscosity where ϕ is the volume of spheres. Sibree (1930, 1931, 1934) tested the equation experimentally on paraffin emulsions and found that the volume ϕ calculated from relative vis-

cosity was in constant ratio (1.3) to the volume of particles determined from photomicrographs of the same emulsions.

In Figure 8, the volume of starch granules determined by centrifuging is compared with the volume calculated from relative viscosity at the same concentration. Although the curves tend to level off at higher concentrations, it can hardly be said that they reach a constant value. Gallay (1936) has discussed this relationship and finds a constant ratio of 0.8 between the volume of granules (calculated from viscosity at the critical concentration) and the reciprocal of the critical concentration (where he believes the granules affect the whole volume of solution) for a series of acid-modified corn and potato starch pastes.

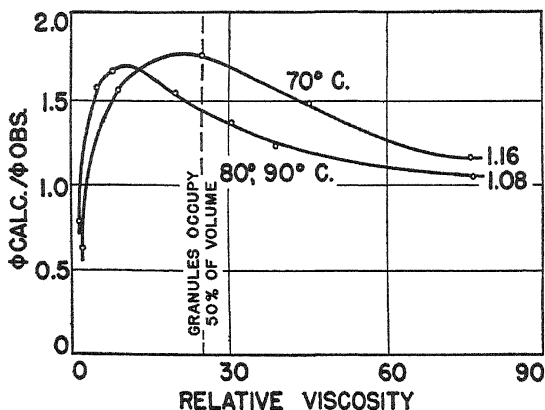


Fig. 8. Ratio of volume of granules (ϕ) observed by centrifuging to that calculated from viscosity by the Hatschek-Sibree equation.

Mark and Simha (1940) have recently reviewed the progress made in the study of nonspherical particles of high polymers with respect to their viscosity and molecular structure.

Logarithmic expressions are sometimes used for viscosity-concentration relationships. Rask and Alsberg (1924) compared a series of wheat starches by plotting the logarithm of viscosity against concentration to give a straight line with the equation:

$$\log y = \log b + mx \quad (7)$$

where y = viscosity of the suspension and x = concentration of starch. Each starch was characterized by the constant m , the slope of the line, and $\log b$, the intercept on the concentration axis, which is frequently the viscosity of the solvent itself. This type of equation, proposed originally by Arrhenius in the form:

$$\eta_s/\eta_0 = C \log A, \quad (8)$$

has since been applied to various colloidal systems. For example, Traxler, Schweyer, and Moffatt (1937), using the exponential form:

$$\eta = C10^{AF}, \quad (9)$$

found that it fitted their viscosity data on asphalt-mineral powder suspensions.

In Figure 9, viscosity-concentration data for unmodified cornstarch pastes prepared at 90°, 80°, and 70° are treated according to the method described above. The fan-shaped group of curves shows that the method must be used with caution. In this case straight lines are

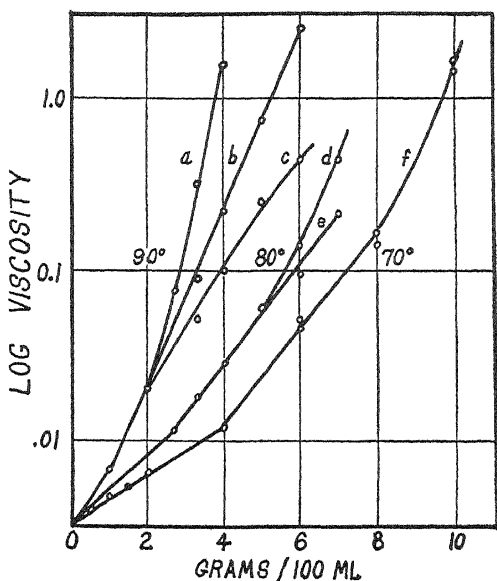


Fig. 9. Application of the Rask-Alsberg method to starch pastes at different temperatures and pressures: a, 15 cm H₂O pressure; b, 75 cm; c, 275 cm; d, 44 cm; e, 140 cm; f, 85 and 135 cm.

obtained only at certain ranges of concentration and pressure. At low pressures, the logarithm of viscosity increases more rapidly than the concentration, giving curves convex to the viscosity axis. This tendency is even more pronounced at 70° than at 90°. Morgan and Vaughn (1942) report this effect with slightly dextrinized starch mixtures. Similar curves for waxy maize starch pastes were inclined to slope in the opposite direction, perhaps because of a crowding and breaking effect on the more susceptible granules as concentration and rate of shear increased.

Discussion

Industrial control of starch products is generally based on the measurement of flow properties, the fluidity funnel being the commonest type of instrument. A comparison of the latter with rotational and capillary type viscometers has been made by Morgan and Vaughn (1942) and Gallay and Bell (1936). Despite the widespread use of viscosity methods, many starch workers are under the impression that such measurements cannot be made on starch pastes with any degree of precision. It is hoped that this idea will be dispelled by the evidence which is accumulating against it, particularly in the publications of the British Textile Institute. Richardson and Waite (1933) state that if the conditions of flow are standardized either by specifying the shearing stress or the rate of shear at which a measurement is made, the same value of apparent viscosity of a paste is obtained from measurements made in different capillaries. The work of Farrow, Lowe, and Neale (1928) is especially convincing in that flow values from both rotational and capillary viscometers could be plotted on the same straight line (log shear—log stress). Duplication of results by different operators using the fluidity funnel has also been demonstrated (Morgan and Vaughn, 1942). But more important than the method of measuring flow is accurate control during preparation of the paste—it should be allowed to attain equilibrium under the conditions chosen.

Quite independent of the inherent composition of the starch, such factors as the following influence the apparent viscosity of its pastes: (1) aggregates of granules which surround portions of the liquid medium and behave as a unit; (2) granules which have swollen sufficiently to become deformable under pressure and no longer act like rigid spheres; (3) empty sacs of granules which have collapsed; and (4) soluble material which has diffused out of the granules and changed the composition of the dispersing medium. It is apparent that no one mathematical expression could be expected to account for the response of each of these factors to changing pressure, temperature, time, concentration, and the mechanical factors operating during preparation of the colloidal system.

There have been proposed a number of viscosity methods which involve the presence of added reagents. Some of them attempt to subordinate the gross colloidal factors of the system to the factors more inherent to the starch itself. A recent example is "thiocyanate viscosity" (Richardson, 1939; Jambuserwala, 1941) in which the starch is homogenized and dispersed in calcium thiocyanate. In other methods, the added reagent is intended only to stabilize the viscosity, such as formaldehyde (Farrow, Lowe, and Neale, 1928). The soap-starch viscosity procedure (Houtz, 1941; Heald, 1941) supposedly augments

structural viscosity under the proper conditions and is reported to give a good indication of strength which the starch will impart when used as a paper size.

When it comes to a study of the starch itself, such methods all have the disadvantage that another variable (added reagent), whose effect is not thoroughly understood, is added to an already complex system of many variables.

Hot viscosities have been used throughout this paper in order to avoid the added complications introduced by cooling such systems. Although a clear picture of what happens when a starch paste is cooled cannot be given from the present state of knowledge, the authors' concept is stated as follows:

When a starch paste is cooled, portions of it tend to revert to a more insoluble form, a phenomenon often included under the terms *aging*, *retrogradation*, or *crystallization*. It is accompanied by an increase in opacity of the paste. If retrogradation is accelerated by cooling or freezing, the retrograded starch can be shown by X-ray diffraction measurements (Bear and French, 1941) to be at least in part crystalline. Starches differ markedly in their tendency to retrograde, waxy maize being especially resistant to crystallization. Within a starch itself, one fraction (amylose) retrogrades easily, in contrast to the amylopectin fraction. This difference in behavior may be associated with the straight-chain or branched structure of the molecule.

It seems logical to explain the nonfluid condition of starch gels as caused by an interlacing of crystalline starch fragments between the aggregated starch granules as well as within the granules themselves. It has been shown that starch gels of sufficiently high concentration are true solids rather than colloidal liquids and exhibit true elasticity (Hixon and Brimhall, 1941).

The preceding paragraphs carry the implication that viscosities should be measured on hot pastes rather than on cold pastes to avoid the variations in crystallizing tendency shown by different starches and starch fractions. The suggestion might be made that the effect of cooling is not merely to increase the degree of structural viscosity by causing aggregation, but that it causes a change in the physical character of the starch itself (crystallization), which in high enough concentrations results in its assuming a solid, elastic character. The degree of elasticity may be used to characterize the cooled gel and measured as a property distinct from viscosity (Brimhall and Hixon, 1939). A clearcut illustration of this is provided by a comparison of the rigidity and viscosity of waxy maize starch with that of ordinary cornstarch, the former having very high hot viscosity but almost no tendency to set to a gel.

Summary

The various methods for interpreting viscosity measurements as applied to starch pastes are reviewed. The application of these methods is illustrated with data obtained by the authors at different temperatures, concentrations, and pressures, with a capillary viscometer.

It is pointed out that starch paste is a heterogeneous system, each constituent of which may react differently toward changes in the conditions of measuring viscosity. The extreme susceptibility of the colloidal state of starch to variations in the method of preparation is stressed. These induced changes are such that no one mathematical expression could be expected to represent flow characteristics over a very wide range.

Measurement of hot viscosity rather than cold viscosity is recommended to avoid the additional complications introduced by cooling the paste. The quantitative determination of gel strength or elasticity as a measure of the solid characteristics of a starch is considered to be a valuable adjunct to viscosity, which is a measure of its fluid characteristics.

Literature Cited

- Barr, Guy
1931 A monograph of viscometry. Oxford University Press, London.
- Bear, R. S., and French, D.
1941 The significance of X-ray diffraction patterns obtained from starch granules. *J. Am. Chem. Soc.* **63**: 2298-2305.
- Bingham, E. C.
1922 Fluidity and plasticity. McGraw-Hill, New York.
1930 Some fundamental definitions of rheology. *J. Rheol.* **1**: 507-516.
- Briggs, C. A., and McCarthy, J. L.
1942 Starch evaluation by a new viscosity method. *Paper Trade J.* **114** (No. 4): 37-40.
- Brimhall, B., and Hixon, R. M.
1939 The rigidity of starch pastes. *Ind. Eng. Chem. (Anal. Ed.)* **11**: 358-361.
- Caesar, G. V.
1932 Consistency changes in starch pastes. *Ind. Eng. Chem.* **24**: 1432-1435.
— and Moore, E. E.
1935 Consistency changes in starch pastes. *Ind. Eng. Chem.* **27**: 1447-1451.
- Farrow, F. D., and Lowe, G. M.
1923 The flow of starch pastes through capillary tubes. *J. Textile Inst.* **14**: 414-440.
- Farrow, F. D., Lowe, G. M., and Neale, S. M.
1928 II. The flow of starch pastes—flow at high and low rates of shear. *J. Textile Inst.* **19**: T18-31.
- Gallay, W.
1936 The effect of concentration on the viscosity of starch pastes and of lyophilic sols. *Can. J. Research* **14B**: 391-403.
— and Bell, A. C.
1936 The viscosity of cornstarch pastes. *Can. J. Research* **14B**: 360-372.
- Harrison, W.
1911 On some properties of starch relating to its stiffening power. *J. Soc. Dyers Colourists* **27**: 84-88.
- Hatschek, Emil
1913 The general theory of viscosity of two-phase systems. *Trans. Faraday Soc.* **9**: 80-92.

- 1928 The viscosity of liquids. Bell & Sons, London.
- Heald, A. M.
1941 Colloidal behavior of soap-starch systems. Paper Trade J. **113** (No. 2): 39.
- Hixon, R. M., and Brimhall, B.
1941 A gelometer for starch pastes. Ind. Eng. Chem. (Anal. Ed.) **13**: 193-194.
- Houtz, H. H.
1941 The colloidal differentiation of starches. Paper Trade J. **113** (No. 6): 32-36.
- Humphrey, Edith, and Hatschek, Emil
1916 The viscosity of suspensions of rigid particles at different rates of shear. Proc. Phys. Soc. London **28**: 274-283.
- Jambuserwala, G. B.
1941 Modified starches. IV. Action of alkalis on starch. J. Textile Inst. **32**: T201-208.
- Katz, J. R., Desai, M. C., and Seiberlich, J.
1938 The change in viscosity of starch pastes explained by the changes in microscopical structure of the starch granules of the paste. Trans. Faraday Soc. **34**: 1258-1268.
- Mark, H., and Simha, R.
1940 Viscosity and molecular structure. Nature **145**: 571-573.
- McDowell, C. M., and Usher, F. T.
1931 Viscosity and rigidity in suspensions of fine particles. I. Aqueous suspensions. II. Non-aqueous suspensions. Proc. Royal Soc. London **131A**: 409-427; 564-576.
- Morgan, W. L., and Vaughn, N. L.
1942 Starch viscosity or strength. To be published in Ind. Eng. Chem.
- Nivling, W. A.
1924 Starches, their fluidity and viscosity in relation to sizing value for textiles. Barr-Erhardt, New York.
- Philippoff, W.
1935 Zur Theorie der Strukturviskosität, I. Kolloid-Z. **71**: 1-16.
1936 Die Bedeutung der Viskosität für die Chemie der Cellulose. Cellulosechem. **17**: 57-77.
- Porst, E. G., and Moskowitz, M.
1922 Comparison of the various Corn Products Co. starches as shown by the Bingham-Greene plastometer. Ind. Eng. Chem. **14**: 49-52.
- Rabinowitsch, B.
1929 Über die Viskosität und Elastizität von Solen. Z. physiol. Chem. **145A**: 1-26.
- Radley, J. A.
1940 Starch and its derivatives (pp. 39-46). Van Nostrand, New York.
- Rask, O. S., and Alsberg, C. L.
1924 A viscosimetric study of wheat starches. Cereal Chem. **1**: 7-26.
- and Waite, R.
1933 The flow of starch pastes. iii. The effect of soaps and other electrolytes on the apparent viscosity of hot starch pastes. J. Textile Inst. **24**: T383-416.
- Richardson, W. A.
1939 A proposed viscosimetric method for the characterization of soluble starch. Chem. and Ind. **1939**: 464-465.
- St. John, J. L.
1927 A simple capillary-tube plastometer. Ind. Eng. Chem. **19**: 1348-1349.
- Samec, M., and Blinc, M.
1937 Methodik der Viskositätsmessungen von Starkekleistern und Starkelösungen. Vth Congres intern. tech. chim. ind. agr., pp. 214-249, Sec. 15.
- Scott Blair, G. W., and Crowther, E. M.
1929 The flow of clay pastes through narrow tubes. J. Phys. Chem. **33**: 321-330.
- Sibree, J. O.
1930 Viscosity of emulsions. Trans. Faraday Soc. **26**: 26-36.
1931 Viscosity of emulsions. *Ibid.* **27**: 161-176.
1934 The viscosity of froth. *Ibid.* **30**: 325-331.
- Traxler, R. N., Schwyer, H. E., and Moffatt, L. R.
1937 Viscosities of liquid-solid systems. Ind. Eng. Chem. **29**: 489-492.

DEXTRINS: THE RELATION OF FERMENTABILITY TO SOLUBILITY AND REDUCING POWER

ELMER F. GLABE

Stein, Hall Manufacturing Co., Chicago, Illinois

(Read at the Annual Meeting, May 1941)

The starch factor in flour and bread has recently received much attention from cereal chemists. The susceptibility of starch to the attack of amylolytic enzymes is a problem the solution of which is fundamental in appreciating the complicated role which starch plays in a flour-water-yeast system.

Raw starch cannot be used by yeast in the manufacture of carbon dioxide gas. It must first be placed in proper condition for conversion to sugar by enzymes normally found in flour. Now it has been found that starch which has been damaged, by milling for example, is more readily attacked by these amylolytic enzymes. Apparently, milling produces starch-breakdown products, which, being of a lower order of complexity, lend themselves more readily to further reduction by enzymes. These changes in susceptibility have been measured by Newton, Farley, and Naylor (1940).

Familiar among the products of starch decomposition are the dex-
trins. They are those interesting carbohydrate compounds, ranging between raw starch and sugar, which are as yet relatively unexplored. Because of their complex nature little is known of their structure. Their behavior in dough fermentation should largely depend upon their rate and degree of conversion to fermentable sugars by the flour amylases. Kent-Jones and Amos (1940) showed that the quantity of dex-
trins in bread dough is a factor in the handling and baking of such a dough, and that this factor influences the character of the crumb in the finished bread. These workers devised a quantitative test for dextrin in flour.

There is, however, another factor worthy of consideration and that is the character of the dextrin. This investigation has been conducted on commercially available dex-
trins to demonstrate that these sub-
stances vary greatly in composition and in effect on fermentation.

Commercial dex-
trins are manufactured by heating pure starch in the presence of small quantities of mineral acids. The chemical make-up of these dex-
trins changes continuously under this treatment. It is possible therefore to get a host of different mixtures of these com-
pounds known as dex-
trins, merely by varying the heating time and/or the acid (Bauer, 1912). For example, it has been known for some time that the percentage of soluble material in the dextrin varies with the

treatment time and the amount of acid used. This has been noted by Schoch (1941). In commercial practice, this figure is often used as a measure of dextrinization. The reducing values of dextrans also change with the type of treatment.

In this paper we shall attempt to show the relationship in dextrans of fermentability to reducing value, on the one hand, and to solubility on the other.

Methods

In this investigation ordinary flour-water-yeast doughs were employed to demonstrate fermentability. The dextrans were added to these doughs by dry-mixing them with the flour. Aliquot portions of these doughs were carefully weighed off and placed into a pressure-meter apparatus similar in principle to, but differing in design from, that employed by Sandstedt and Blish (1934) described by Glabe (1942, Fig. 1). The amount of gas evolved was recorded at hourly intervals up to 4 hours and then again at 4½ hours. The values obtained were then calculated to the base of 100 g of flour at 15% moisture at 760 mm of pressure and 30°C. The figures obtained at the 4½-hour period were used for comparing fermentabilities.

Solubility was determined by stirring a given quantity of dextrin in water, filtering, and determining the specific gravity of the filtrate. The specific gravity gives a measure of the amount of soluble material in the dextrin. Reducing values were determined by the Schoorl volumetric method.

Increase in Gas Production by Dextrin

In the first step the relationship of the reducing value of the dextrin to its gas-producing ability was determined. A popular short patent flour which had been lightly malted and bleached was used as a substrate. The effect upon gas production of adding to this flour 1%, 2%, and 3% of a commercial corn dextrin was compared with the effect produced by adding similar quantities of cp dextrose, maltose, and sucrose. The results are shown in Table I. The dextrin upon analysis showed a reducing value of 7.93% calculated as dextrose. For all three percentages the dextrin, because of its amylase susceptibility, showed a larger increase in gas over that produced by the control than would have been expected from its reducing value.

A second series of tests with the same flour showed that whereas 3% of dextrose added to the flour produced 2,225 ml of gas per 100 g of flour, and 3% of dextrin produced 1,890 ml of CO₂, a quantity of dextrose equivalent to the reducing value of 3% of the dextrin produced only 1,620 ml. The flour with no additions of dextrose or

TABLE I

DEXTRIN SERIES I—EFFECT ON GAS PRODUCTION OF ADDITIONS OF COMMERCIAL CORN DEXTRIN TO DOUGH AS COMPARED TO ADDITIONS OF EQUAL QUANTITIES OF DEXTROSE, SUCROSE, AND MALTOSE

Substance added	CO ₂ produced in 4½ hours by 100 g flour		
	1%	2%	3%
	ml	ml	ml
Dextrin	1,635	1,735	1,890
Dextrose	1,825	2,030	2,225
Sucrose	1,905	2,065	2,210
Maltose	1,825	2,020	2,225
Gas production of the flour	1,550 ml		

dextrin showed a level of 1,560 ml. This again is attributable to saccharification of the dextrin by flour amylases.

Since solubility, as mentioned before, is used as a measure of dextrinization in commercial practice, this factor might be found to bear some relationship to differences in fermentability.

Solubility vs. Gas Production

A series of three commercial corn dextrans of varying solubilities was studied in an attempt to correlate solubility with fermentability. This is dextrin series II.

In this series of tests two hard winter wheat flours were employed. Both were of the same extraction, but flour A was unbleached and unmalted, and B was unmalted but bleached. The maltose value for flour A was 275 and for flour B 268. To these flours were added 0.1% of commercial malted wheat flour in conjunction with varying quantities of the dextrans.

Table II gives the characteristics of the dextrans of series II and the results for total gas produced by adding 3% of the dextrans to unbleached flours A and B plus 0.1% of malted wheat flour. It will be observed that although the solubility of dextrin No. 3 was greater than that of No. 1 and No. 2 it produced less gas than either. The reducing value of dextrin No. 3 was also slightly greater than that of No. 2, yet the gas produced by dextrin No. 3 was less than that produced by No. 2. Apparently this slight advance in reducing value is of no consequence in the light of relative gas-production values.

Relatively similar results were obtained when the bleached flour B was used, although the amount of gas produced by each dextrin was slightly less than when unbleached flour was used. The values seem to indicate a relationship of some sort between "degree of dextrinization" and fermentability or condition for saccharification by enzymes.

TABLE II

DEXTRIN SERIES II—EFFECT ON HARD WHEAT FLOUR OF ADDITIONS OF 0.1% OF MALTED WHEAT FLOUR AND 3% OF COMMERCIAL CORN DEXTRINS OF VARYING SOLUBILITY

Dextrin No.	Soluble material	Reducing value as % dextrose	CO ₂ produced in 4½ hours by 100 g flour	
			Unbleached flour A	Bleached flour B
	%	%	ml	ml
1	30	4.12	1,980	1,910
2	60	8.03	2,060	1,975
3	80	8.28	1,860	1,855
Flour gas production	—	—	1,730	1,690
Flour plus malt flour	0.1	—	1,775	1,750

In order to explore this idea, commercial dextrin runs were made in which 1400 lbs of corn starch was heated at 265°–270°F in the presence of 3500 ml of hydrochloric acid at 10 Bé with constant agitation. Samples were drawn off at regular intervals during a four-hour treatment in order to obtain a set of dextrans varying in solubility from low to extremely high percentages. These dextrans comprise group III.

Samples were tested for solubility, reducing power, and fermentability at 3% increments as previously. The upper curves of Figure 1 show fermentability or gas-production values plotted against solubility of the dextrans of series III. The solid line represents the total gas produced with unbleached flour; the dotted line gives the results with bleached flour. The lower curve represents reducing power (as percent of dextrose) plotted against solubility. It will be noted that the first appreciable increase in gas production over the control was found when the solubility of the dextrin reached 4.0%. Fermentability increased up to the solubility of 69%. Thereafter fermentability decreased as the solubility rose. It is immediately apparent that the gas-production values of this series of corn dextrans are more a function of the solubility than of reducing power, since gas production begins to fall even though reducing values continue to increase.

The extreme right-hand portion of the fermentability curve also shows an interesting characteristic. The last three dextrin samples which were collected had solubilities of 100%. The last two dextrans apparently had a singular effect on fermentation, since the gas-production values as indicated by the curve were less than the value of the control, and this in the face of the fact that they showed a reducing value of more than 5%. The curve of the bleached flour shows a

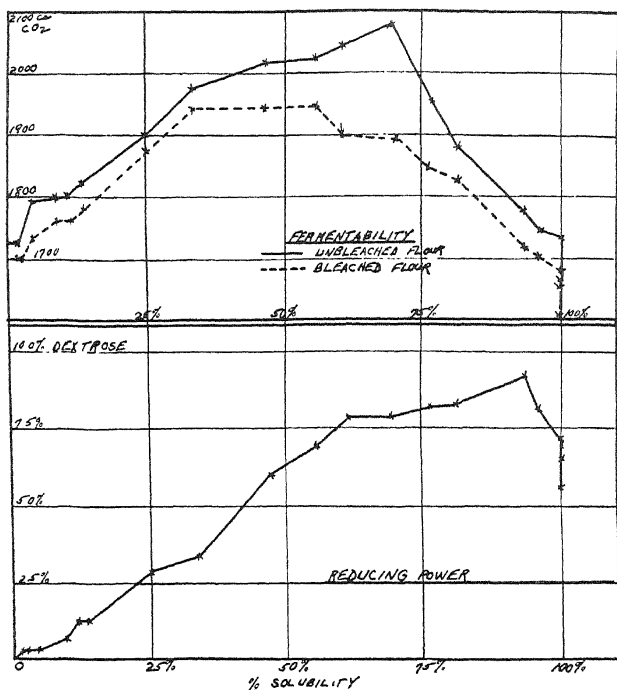


Fig. 1. Dextrin series III—fermentability vs. solubility and reducing power.

very interesting point in that the highest fermentability is reached at 56% solubility as against the highest fermentability with the unbleached flour of 69%.

Summary

Several series of dextrins made from acid-hydrolyzed corn starch yielded increases in gas production of fermenting doughs not always compatible with their reducing-power values. These corn dextrins increased in fermentability and susceptibility to amylase attack as their solubility in water rose to 69%, thereafter suffering a rapid decline. At the ideal solubility the amount of gas increase produced by these corn dextrins was many times that which would be produced by a quantity of dextrose of equivalent reducing value, indicating that this is the point of maximum amylolytic conversion.

There were some indications that the dextrinization of corn starch, when continued until the starch was 100% water soluble, produced material which was not fermentable, even after exposure to amylases.

Reducing power in dextrins is not always a true indication, either of the extent of dextrinization or of fermentability.

Literature Cited

- Bauer, H. F.
1912 Eighth international congress of applied chemistry 13: 9.
- Glabe, E. F.
1942 The fermentometer—a gas-production measuring device; the effect of varying pressure on gas production in dough. *Cereal Chem.* 19: 230–234.
- Kent-Jones, D. W., and Amos, A. J.
1940 A new flour test: the dextrin figure. *Cereal Chem.* 17: 265–278.
- Newton, J. M., Farley, F. F., and Naylor, N. M.
1940 The use of soybean beta-amylase to follow the modification of starch. *Cereal Chem.* 17: 342–354.
- Sandstedt, R. M., and Blish, M. J.
1934 Yeast variability and its control in flour gassing power tests. *Cereal Chem.* 11: 368–383.
- Schoch, T. J.
1941 Physical aspects of starch behavior. *Cereal Chem.* 18: 121–128.
- Schoorl, N.
1935 Volumetric method for reducing sugars. *Cereal Laboratory Methods: American Association of Cereal Chemists.*

THE NATURE OF THE INTERFERING CHROMOGENS ENCOUNTERED IN THE DETERMINATION OF NICOTINIC ACID IN CEREAL PRODUCTS

ELMER B. BROWN, JAMES M. THOMAS, and ALBERT F. BINA

Anheuser-Busch, Inc., Laboratories, St. Louis, Missouri

(Received for publication December 22, 1941)

Since the establishment of the importance of nicotinic acid and nicotinic acid amide in nutrition, a number of investigators have reported work carried out on various materials. The primary object of these investigations was to determine the nicotinic acid content of the specific material in which the investigator was interested, and hence to develop a suitable procedure applicable to the specific material. Most of the material investigated was of animal origin and the problems encountered were usually specific for this type of product. As a result several procedures have shown sufficient promise in the work to which they were applied to suggest their use to workers interested in the nicotinic acid content of other products. When these different procedures are used for the estimation of the nicotinic acid content of any one product, the results obtained, as a rule, are different. Most of the investigators recognize that their procedures are limited to specific material and are not of general application.

The varying results reported for the amounts of nicotinic acid in cereal products by the application of different procedures has caused

confusion in regard to the actual nicotinic acid content of cereals. At the government hearing on bread standards in Washington, data were supplied on the nicotinic acid content of wheat, various grades of flour, and bread. These results were two to three times higher than the amounts of nicotinic acid that we have been able to find in comparable grades of flour and bread. They were also not reconcilable with the fact that such materials are low in nicotinic acid when tested by animal experimentation for black tongue. The chemical methods by which these results were obtained were not stated.

Bina, Thomas, and Brown (1941) developed a procedure for the determination of nicotinic acid in cereal products that gave consistent results on all products to which it was applied. Thomas, Bina, and Brown (1942) applied this method to yeast, bread, and other cereal products and showed that wheat and flour do not contain the high amounts of nicotinic acid that are indicated by some of the other procedures, but showed amounts in line with the fact that these substances are low in nicotinic acid. These authors also pointed out that interfering substances that produce high values are likely to be present in the extracts from cereal products, and that the procedure must be designed to avoid such interferences. Since the procedure of Bina, Thomas, and Brown was designed to eliminate this interference, it did not include any discussion of the nature of the interfering substances.

All of the procedures suggested for the determination of nicotinic acid are based on the method of König, in which cyanogen bromide is used to react with the nicotinic acid, and the reaction product in the presence of a suitable aromatic amine combines to produce a color reaction that can be measured. The color developed is compared to the color produced with a known amount of nicotinic acid treated similarly. The procedures differ in the preparation of the extract, the method selected for making the extract preparatory to color development, the aromatic amine used, and the technique employed in the color development.

All of these steps are important and it will be shown in the experimental part of this paper that an interfering substance is produced causing abnormally high results in cereal products when determined by the procedure of Melnick and Field (1940). We find that this interfering substance is developed, in the hydrolysis step of their procedure, by the treatment with concentrated hydrochloric acid and is not removed by their subsequent treatment. The decolorizing technique with charcoal which these authors employed for decolorizing their extract has no effect on the removal of this substance, and it remains in the solution to give interference by their procedure of color development with cyanogen bromide and aniline. The fact that the

color produced by this compound, cyanogen bromide, and *p*-aminoacetophenone is not extractable with ethyl acetate shows the importance of the ethyl acetate step in the procedures that take advantage of this selective solvent to extract the color produced by these reagents in the nicotinic acid determination. Furthermore, it is shown that when this interfering substance is removed or avoided in the preparation of the extract the nicotinic acid content of cereal products becomes identical with the results obtained by Thomas, Bina, and Brown (1942).

A further point of interest is the fact that this interfering substance is not encountered in the determination of nicotinic acid in yeast products by the use of concentrated hydrochloric acid in the hydrolysis.

Waisman and Elvehjem (1941), using essentially the procedure of Melnick and Field (1940), report a value obtained on yellow corn of 10.7 mg per 100 g or 48.47 mg per pound. Since this material was used in their routine production of black tongue in dogs, they concluded that the presence of some unknown chromogen other than nicotinic acid was responsible for the high value and that this substance does not possess antipellagric properties.

Dann and Handler (1941) report values for nicotinic acid in samples of yellow corn which are in close agreement with results we obtain. They found 6 to 10 μ g per gram on these samples, corresponding to 2.7 to 4.5 mg per pound. This compares with 3.17 mg per pound on the sample we analyzed. These authors remove the nicotinic acid from the extract (and thereby free it from the compounds not removed) by Lloyd's reagent. Since Lloyd's reagent also removes all of the alkaloidal substances and coloring material present in the extract along with the nicotinic acid, they further treat with lead nitrate the colored solution produced on dissolving the Lloyd's precipitate in caustic. This produces a colorless solution containing the nicotinic acid which these authors deem essential for their color development with metol.

Kodicek (1940) reports values for nicotinic acid obtained on yellow corn ranging from 6 to 27 μ g per gram, dependent upon whether the determination was made on the aqueous extract subsequently hydrolyzed with sodium hydroxide, or the extraction was made originally with 8% of sodium hydroxide solution. This difference was not due in his opinion to imperfect extraction but to some unspecific chromogen which was not in fact a pellagra-preventing substance. This author tested this corn by feeding to dogs and found they all developed black tongue in the usual period of time. From the negative biological results obtained he concluded that the water extract which showed 6 μ g per gram provided the true value for this substance and that extraction with boiling water permits the quantitative extraction of active nico-

tinic acid. Similarly, wheat germ gave considerably higher values when the extraction was made with 8% sodium hydroxide (39 μ g per gram) than with boiling water (27 μ g per gram). It is to be observed that Kodicek used *p*-aminoacetophenone in this work for the color development. He did not employ ethyl acetate to extract the color complex developed where interfering substances are present, as recommended by Harris and Raymond (1939).

Experimental

In order to establish the cause of the wide differences in results obtained on various cereal products by different chemical procedures of analysis for nicotinic acid, experiments were planned to include determination of the nicotinic acid content of the same materials by different procedures.

Yeast: A sample of regular bakers' yeast was used for the first set of experiments. The nicotinic acid content of this material was determined by the procedure of Bina, Thomas, and Brown (1941) and of Melnick, Oser, and Siegel (1941). The results obtained on this yeast gave 0.391 mg of nicotinic acid per gram by the first procedure and 0.394 mg per gram by the second procedure.

An enriched yeast was next used for the determination by the two procedures. 515.0 mg per pound was obtained by the Bina, Thomas, and Brown procedure and 520.4 mg per pound by the Melnick, Oser, and Siegel procedure. These results are practically identical and show that no substance was encountered that affected the determination of nicotinic acid in yeast by either procedure. The values are also in line with the amounts of nicotinic acid that are expected to be present in the two samples of yeast.

Whole-wheat flour: Whole-wheat flour was used for this set of experiments and nicotinic acid was determined by the same procedures as with yeast. The results obtained, however, were entirely different by the two methods, as this flour showed 7.44 mg of nicotinic acid per pound by the Bina, Thomas, and Brown procedure and 21.47 mg per pound by the Melnick, Oser, and Siegel procedure.

Since the extract prepared by the latter took on considerable color in the treatment and contained alcohol, the remaining solution, from which the above aliquot was taken, was evaporated to dryness on the water bath, treated with 15 ml of concentrated nitric acid, and again carried to dryness. This treatment with nitric acid was repeated twice more to oxidize as much of the organic material as possible before finally diluting with water and evaporating to remove the nitric acid. The residue was then made up to volume with water and alcohol for further analysis. One aliquot of this retreated extract was used for

the determination of nicotinic acid by the Melnick, Oser, and Siegel method. The results show that the blank value had been reduced considerably but the apparent nicotinic acid value of the sample had been slightly increased by the treatment. A value of 22.98 mg per pound was obtained on this retreated extract as compared to 21.47 originally determined.

A second aliquot of this solution was used for the determination of nicotinic acid by the Bina, Thomas, and Brown procedure. This method employs *p*-aminoacetophenone instead of aniline in the color development, and the color complex produced is extracted with ethyl acetate so that the readings can be made on the ethyl acetate solvent instead of the original extract as is the case in the Melnick, Oser, and Siegel procedure. The results showed 8.29 mg per pound on this extract against 7.44 mg per pound as determined on the original material by this method. It is apparent that the nitric acid treatment did not remove any of the color affecting the determination even though the blank values were considerably reduced. It is also evident that this extract contains a large amount of material other than nicotinic acid that produces a color complex with aniline, and consequently is determined as nicotinic acid by the Melnick, Oser, and Siegel procedure. This color complex is not extractable with ethyl acetate in the Bina, Thomas, and Brown procedure and, therefore, is eliminated from the nicotinic acid measurement by that method.

Dann and Handler method: A sample of the same whole-wheat flour was subjected to hydrolysis and treatment according to the procedure of Dann and Handler (1941) for the determination of nicotinic acid. In this procedure the acid hydrolysis is made on the sample instead of on the aqueous solution, with a relatively high concentration of hydrochloric acid and at boiling-water-bath temperature for two hours. The extract was next treated with Lloyd's reagent to adsorb the nicotinic acid and the precipitate was washed with 0.2 *N* sulfuric acid and redissolved in caustic so that it could be decolorized with lead nitrate according to prescribed procedure. A water-clear, practically colorless solution was obtained. This solution was then treated with cyanogen bromide and metol according to the color production technique of this procedure. The color produced, however, was dark brown instead of the characteristic lemon yellow, and therefore not readable in the fluorophotometer.

Another sample was hydrolyzed and treated by the same procedure up to the point of color development. One aliquot of this solution was treated with aniline and cyanogen bromide according to Melnick, Oser, and Siegel and the other with *p*-aminoacetophenone according to Bina, Thomas, and Brown's color technique. The results showed

22.52 mg per pound by the Melnick, Oser, and Siegel method and 8.13 by the Bina, Thomas, and Brown procedure. These results, where the procedures used for extracting and hydrolyzing the samples differed, are not materially different from the preceding results where the procedures used were in accordance with the authors' recommendations.

Action of oxidizing agent on the interfering chromogen: 20 g of the same whole-wheat flour as previously used was hydrolyzed according to the procedure of Dann and Handler, with relatively concentrated hydrochloric acid. This extract was made up to a known volume and aliquots used for further treatment. This extract was highly colored and contained considerable chromogen, other than nicotinic acid, that produces color with cyanogen bromide and aniline according to the procedure of Melnick and co-workers.

Ten ml of this solution was treated with 5 ml of strong hydrogen peroxide by placing both in a test tube and heating in a water bath at 60°–70°C as long as oxygen was given off. The water-bath temperature was then brought to the boiling point for a half hour to remove any excess hydrogen peroxide from the acid solution. The hydrogen peroxide decolorizes the solution to give a water-clear extract. This solution was neutralized and the nicotinic acid determined with aniline according to the Melnick, Oser, and Siegel procedure, the results being compared to the original extract. The apparent nicotinic acid content was reduced from 23 mg per pound on the original extract to 13 mg per pound by this treatment.

Another 10-ml portion of the extract was treated with hydrogen peroxide to give a colorless extract as above, and then concentrated to dryness on the steam bath to expell the excess hydrochloric acid. The residue was dissolved in water and gave a strongly colored solution as a result of evaporation with the acid. This solution was again decolorized by treatment with hydrogen peroxide as before. To the colorless solution, which showed only slight acidity, was added Lloyd's reagent to remove the nicotinic acid so that the remaining purification operations could be carried out by Dann and Handler's method of preparing the extract. The color development was made with aniline by the same method as with the first aliquot. This sample gave a nicotinic acid content of 7.88 mg per pound, a value in good agreement with the results of 7.44 mg per pound obtained by the Bina, Thomas, and Brown method.¹

Furfuraldehyde: The successful oxidation with hydrogen peroxide

¹ It is essential that all of the hydrogen peroxide be removed from the solution prior to color development, to prevent oxidation of the reagent. The evaporation of the acid solution produces color that should be removed from the solution before color development. It was observed that the excess hydrogen peroxide used in the oxidation is not readily removed from the solution since sufficient amounts are carried over by the Lloyd's reagent to oxidize to the yellow oxide some of the lead nitrate used in the final purification step.

of the interfering chromogen produced by treatment with strong hydrochloric acid or strong caustic solution suggests aldehydes as the cause of this interference. It is well known that aldehydes are readily produced from cereal products by heating with hydrochloric acid and that furfuraldehyde and aniline react to give a colored complex.

Kodicek (1940), as well as others, calls attention to the possible interference from aldehydes, if present, in this reaction, but gives no specific data that would lead one to suspect that aldehydes are the source of this interference. In order to ascertain what effect furfuraldehyde, if present, would make on the determination, tests were made as follows:

A solution of furfuraldehyde was prepared and dilutions made so that varying amounts of this aldehyde could be tested by treatment with cyanogen bromide and the aromatic amines—*aniline*, *metol*, and *p*-aminoacetophenone—as are used in the determination of nicotinic acid.

Furfuraldehyde produces a strong coloration when treated with cyanogen bromide and aniline and is a potent chromogen if present in the extract where aniline is used. A solution containing 1 part in 15,000 gives rise to a large reading on the galvanometer and is read as nicotinic acid in the determinations using this reagent. The same is true with *metol* where a characteristic yellow color is produced that would give interference. Furfuraldehyde does not produce a color complex when treated with cyanogen bromide and *p*-aminoacetophenone, and hence offers no interference with this amine. The blank value and the solution value were identical with *p*-aminoacetophenone.

The fact that furfuraldehyde does not produce a color complex with cyanogen bromide and *p*-aminoacetophenone shows that this aldehyde is not identical with the chromogen responsible for the high results in the Melnick, Oser, and Siegel extract. That chromogen gave a color complex when treated with cyanogen bromide and *p*-aminoacetophenone, but it was not extractable with ethyl acetate.

Wheat germ: A sample of wheat germ was used for the next set of experiments. In these experiments the nicotinic acid content of wheat germ was determined by three different procedures, namely those of Bina, Thomas, and Brown, who employed *takadiastase* and a mild acid hydrolysis for the preparation of the extract and *p*-aminoacetophenone in the color development; Melnick, Oser, and Siegel, who employed concentrated hydrochloric acid for hydrolysis of the extract, and aniline in the color development; and Kringstad and Naess (1939), who employed a mild acid hydrolysis with dilute sulfuric acid for the preparation of the extract, and aniline in the color development. The following results were obtained:

Bina, Thomas, and Brown.	24.13 mg per pound
Melnick, Oser, and Siegel.	36.24 mg per pound
Kringstad and Naess	24.46 mg per pound

The results obtained by the procedures of Bina, Thomas, and Brown and Kringstad and Naess are practically identical but the results obtained by the procedure of Melnick, Oser, and Siegel are considerably higher and contain material other than nicotinic acid.

An aliquot of the extract prepared by the Kringstad and Naess method of hydrolysis was used for color development by the Melnick, Oser, and Siegel procedure of color development and a value of 24.06 mg per pound was obtained. This shows that the color complex responsible for the high value in the Melnick, Oser, and Siegel extract is not present in the extract prepared by the Kringstad and Naess procedure.

Sulfuric acid hydrolysis: Another aliquot of this extract (Kringstad and Naess) was subjected to hydrolysis by adding sulfuric acid to make a 15% solution and heating for two hours in a boiling water bath. Kringstad and Naess found this treatment would convert any nicotinic acid amide in the solution to nicotinic acid. The excess sulfuric acid was removed with barium hydroxide and the nicotinic acid determined as before the treatment. A value of 21.92 mg per pound was obtained. This result shows a small loss of nicotinic acid rather than the creation of an interfering compound in the reaction. A voluminous precipitate of barium sulfate is obtained in the neutralization of the sulfuric acid with barium hydroxide, and it is possible that the small difference in the results can thus be accounted for.

An aliquot of the solution as treated above with 15% sulfuric acid was evaporated to dryness, hydrolyzed with 10 ml of concentrated hydrochloric acid, and the nicotinic acid determined by the Melnick, Oser, and Siegel procedure. Only a slight increase in color development took place with this treatment; a value of 22.65 mg per pound was obtained on this solution compared to 21.92 mg per pound before the treatment.

Nicotinamide: In order to prove that a mild acid hydrolysis, similar to that employed in the procedure of Bina, Thomas, and Brown, is sufficient to convert nicotinamide to nicotinic acid, a sample of pure nicotinamide was analyzed. When 0.4 mg of this amide was subjected to the procedure of Bina, Thomas, and Brown, 0.42 mg of nicotinic acid was obtained. Since the molecular weights of nicotinic acid and nicotinamide are approximately the same, this result shows that the conversion of the nicotinamide to nicotinic acid was complete.

Alkaline hydrolysis: An extract as prepared by the Melnick, Oser,

and Siegel procedure was subjected to alkaline hydrolysis by first neutralizing the solution and then adding barium hydroxide to make a 10% solution. This solution was heated in a boiling water bath for 30 minutes and the barium removed with sulfuric acid. Considerable color developed in the solution, and it was evaporated to dryness and decolorized with nitric acid as previously described. The residue was dissolved in a known amount of water and aliquots were used for the determination of nicotinic acid by the color techniques of different methods. The results show that the previous value of 36.24 obtained by the Melnick, Oser, and Siegel procedure was reduced to 29.24 by this treatment.

The values of 27.80 and 26.27 obtained on this solution by the Kringstad and Naess and the Bina, Thomas, and Brown color techniques were somewhat higher than the values of 24.46 and 24.13 obtained on the extracts when prepared by their respective methods. This treatment removed a considerable portion of the interfering color complex developed in the Melnick, Oser, and Siegel procedure, but not all of it.

Nicotinic acid content of milk powder: Samples of skim-milk powder and whole-milk powder were used for determinations of nicotinic acid by different methods, and the results compared. The dry skim-milk powder gave 12.25, 20.30, and 12.50 mg per pound, respectively, by the Bina, Thomas, and Brown, by the Melnick, Oser, and Siegel, and by the Dann and Handler methods of analysis. The high value by the Melnick, Oser, and Siegel method is similar to that shown in the comparative results obtained on cereal products by their procedure in which interfering chromogens were shown to be the cause of the high results. This is confirmed by the fact that their extract showed a lower value, 14.5 mg per pound, when the color technique of the Bina, Thomas, and Brown procedure was applied.

Whole-milk powder gave correspondingly lower values than the skim-milk powder, because of the high fat content. A value of 9 mg per pound was obtained on this sample of whole-milk powder.

In the analysis of milk products turbidity is encountered unless special precautions are taken to remove the precipitate that continues to deposit after the neutralization of the extract. We find that this interference is removed if the neutralized extract is cooled in an ice bath for a period of 30 minutes and then centrifuged at high speed just prior to color development.

Discussion and Summary of Results

From the results we have obtained by different procedures for the determination of nicotinic acid in cereal products, yeast, and milk,

we have found that the differences obtained are due mainly to the hydrolysis used in the preparation of the extracts. These results show that strong acid or alkaline hydrolysis is not necessary for the complete extraction of the nicotinic acid in cereal products, but on the contrary is detrimental in that the chromogens and other coloring materials are produced that interfere with the nicotinic acid determination. The nicotinic acid content of cereal products is completely extracted by autoclaving a water suspension and subsequently treating it with taka-diastase. Any amide present is completely converted to the acid by a mild acid hydrolysis without the production of interfering chromogens and with a minimum of color in the extract.

Decolorizing techniques employed by the different procedures for treatment of the extracts proved of little advantage to the method. With the exception of hydrogen peroxide none of the decolorizing treatments did more than remove color that was compensated for by the blank. Some of the procedures were more effective than others for producing water-clear extracts. Decolorizing with charcoal was incomplete and ineffective. The decolorizing technique of Dann and Handler with Lloyd's reagent and lead nitrate produces colorless extracts with very small blank values; however, this treatment does not free the extract from interfering chromogens. Hydrogen peroxide is an efficient decolorizing reagent that will remove interfering chromogens as well as decolorize the solution. Its use for this purpose is not recommended, however, because of difficulty in removing the excess peroxide. If extracts of cereal products are properly prepared, satisfactory solutions will be obtained without the use of decolorizing reagents.

Aniline, metol, and *p*-aminoacetophenone were used in these experiments and gave the same nicotinic values for yeast only, where no interfering substances were produced by any of the procedures tried. Aniline and metol were not found suitable for use in the determination of nicotinic acid in cereal products and milk where interfering substances were produced in the hydrolysis with strong acid and alkali. The use of *p*-aminoacetophenone and ethyl acetate gave consistent results on all of these products for the nicotinic acid content, and the values obtained are in accord with the low anti-pellagric properties found by animal experimentation on these products.

These experiments show the presence of an interfering chromogen in extracts of cereal products prepared by strong acid or alkaline hydrolysis. This chromogen is colorless in solution prior to treatment with cyanogen bromide and the aromatic amine. With metol and cyanogen bromide this chromogen produces a dark brown color that masks the lemon yellow color produced by nicotinic acid and this

amine. This substance produces a color with aniline and cyanogen bromide similar to the color produced by nicotinic acid and is read on the fluorophotometer, thus giving rise to misleading results for nicotinic acid where aniline is used. The color complex produced by this chromogen with *p*-aminoacetophenone and cyanogen bromide is not soluble in ethyl acetate and hence is not extracted by this solvent to interfere in the color reading of the nicotinic acid. Lloyd's reagent precipitates this chromogen completely from the extract along with the nicotinic acid and it is brought back into the solution by the caustic. The subsequent decolorizing action of lead nitrate serves only to remove color normally eliminated by the blank and hence reduces high blank values but does not eliminate this chromogen as an interfering substance. This chromogen is oxidized by hydrogen peroxide.

Literature Cited

- Bandier, E., and Hald, J.
1939 A colorimetric reaction for the quantitative estimation of nicotinic acid. *Biochem. J.* **33**: 264-271.
- Bina, A. F., Thomas, J., and Brown, E. B.
1941 The determination of nicotinic acid in bread and other cereal products. *Cereal Chem.* **18**: 661-666.
- Dann, W. J., and Handler, P.
1941 The quantitative estimation of nicotinic acid in animal tissues. *J. Biol. Chem.* **140**: 201-213.
- Harris, L. J., and Raymond, W. D.
1939 Assessment of the level of nutrition. A method for the estimation of nicotinic acid in urine. *Biochem. J.* **33**: 2037-2051.
- Kodicek, E.
1940 Estimation of nicotinic acid in animal tissues, blood and certain foodstuffs. *Biochem. J.* **34**: 712-723.
- Kringstad, H., and Naess, T.
1939 A colorimetric method for the determination of nicotinic acid and nicotinamide in foodstuffs. *Hoppe-Seyler's Z. physiol. Chem.* **260**: 108-118.
- Melnick, D., and Field, H., Jr.
1940 Determination of nicotinic acid in biological materials by means of photoelectric colorimetry. *J. Biol. Chem.* **134**: 1-16.
- Melnick, D., Oser, B. L., and Siegel, L.
1941 Analytical control of nicotinic acid fortification of flour and bread. Read at spring meeting of American Chemical Society in St. Louis.
- Thomas, J., Bina, A. F., and Brown, E. B.
1942 The nicotinic acid content of cereal products. *Cereal Chem.* **19**: 173-180.
- Waisman, H. A., and Elvehjem, C. A.
1941 Chemical estimation of nicotinic acid and vitamin B₆. *Ind. Eng. Chem. (Anal. Ed.)* **13**: 221-225.

THE UTILITY OF MICRO METHODS OF TEST-WEIGHT DETERMINATION WITH HARD RED SPRING WHEAT¹

R. H. HARRIS and L. D. SIBBITT

North Dakota Agricultural Experiment Station, Fargo, North Dakota

(Received for publication January 30, 1942)

Micro methods of analysis are becoming increasingly important to the scientist. Valuable time and material may be conserved by these techniques, and in the field of cereal technology the introduction of micro methods of experimental milling and baking has enabled the technologist to obtain trustworthy data from wheat samples too small to be tested by the older procedures. These methods are especially valuable in nutrition investigations, as well as in preharvest quality surveys, where only small quantities of wheat are available, as in wheat breeding work. The same situation exists when wheat is grown in nutrient cultures where the cost of space and chemicals limits the amount of wheat that can be produced.

In working with these small samples of wheat the authors have found it necessary to devise some method whereby the test weight per bushel could be ascertained without the use of the standard method described in the *Handbook of Official Grain Standards* published by the Agricultural Marketing Service, U. S. Department of Agriculture. This standard method is based upon the use of a quart measure and required more than a quart of wheat before the test may be employed. By definition of the U. S. Department of Agriculture the test weight of a sample of grain is the weight of the volume of grain required to fill level full a Winchester bushel of 2,150.42 cubic inches capacity. This value is very important in wheat grading, as definite limits in test weight exist among the different grades in sound and unmixed wheats. As grade controls the price, test weight is highly significant to the wheat grower and is therefore of interest to the agronomist and cereal technologist when determining varietal effects upon wheat quality. It is also a factor of importance to the flour manufacturer since it is positively correlated with flour yield.

Micro test-weight technique was used by the Agricultural Marketing Service in a preharvest wheat survey in the hard red spring wheat region in the 1939 season and in the hard red winter wheat region in both 1939 and 1940. In this method a small coin envelope was used to hold the wheat. This envelope was held two inches above the top of a cut-down graduated cylinder when filling. The graduate was tapped lightly three times with a stoker before striking off the wheat

¹ Published with the approval of the Director of the Station.

level with the top of the graduate, which was 16 ml in capacity. The wheat was then weighed and the factor 5.0 used to calculate the weight per bushel from the weight in grams of the sample.

Aamodt and Torrie (1934) studied the relationships between the weight ($g \times 20$) of a 4-ml wheat sample and the weight in pounds per bushel as determined by the usual method, except that an Imperial pint measure was employed instead of the official quart kettle. A cut-down 25-ml graduated cylinder was employed to measure the wheat. The grain was poured into the measure from a coin envelope, pressed down lightly with the thumb, and leveled off with a scalpel drawn across the edges of the measure. It was found that this micro method yielded a close approximation to the test weight per bushel. Correlation coefficients of $+0.947$ for 184 samples of spring wheat and $+0.834$ for 59 samples of winter wheat were obtained between the values found by the two methods. The authors pointed out the utility of the micro method in determining test weight in experiments dealing with small samples harvested from pot cultures in plant nutritional and disease studies and in plant breeding investigations. Weight per 1,000 kernels lacked reliability when different-sized wheat kernels were involved, and the determination was too tedious and expensive when a large number of samples were to be tested.

Harris and Sibbitt (1941) described a procedure for determining test weight in which 4-ml and 16-ml measures were used. These measurements were compared with the test weight obtained by the standard official method using the quart kettle. Lower values were noticeable in the results obtained when the weight in grams of 4 ml of grain was multiplied by the conversion factor of 20 used by Aamodt and Torrie, despite the fact that the bushel in use in Canada contains 36,369 ml, while the Winchester bushel employed in the United States contains only 35,239 ml.

No doubt the discrepancy found between the 4 ml and the standard-method results by Harris and Sibbitt and those reported by Aamodt and Torrie is due to differences in technique in making the micro determinations. The data appeared to indicate the possibility of successfully using micro methods in accurately determining the test weight per bushel of hard red spring wheats, and pointed to the advisability of further investigations of the methods employed with the end of devising by statistical methods a suitable factor or formula to employ in calculating test weights from micro methods. Because of the small number of observations (triplicate tests on ten samples) reported in the preliminary study, it would also be necessary to obtain more comprehensive data before suitable formulas could be worked out.

Materials

The first experiment undertaken was set up to determine the relative degrees of decision in predicting test weight per bushel from the following measurements carried out on very small samples of wheat: (1) weight in grams of 16-ml and 4-ml portions of the wheat, (2) weight of 16-ml and 4-ml portions of ground wheat meal, and (3) weight of 500 kernels.

It was also thought desirable to find out whether there is an "operator" effect—in other words, whether the method in the hands of one worker would yield significantly different results from those obtained by another person. Twenty-two samples of wheat from the field-plot variety trials were included in the series used in this part of the study. These wheats consisted of a number of hard red spring varieties. Thirty samples were also included from the Crop Weather Studies which are carried on at this Station in cooperation with the Agricultural Marketing Service. The latter group consisted of three varieties only—Thatcher, Ceres, and Premier. All tests carried out on this series of wheat were done in triplicate.

A second series, consisting of regular field-plot variety and other miscellaneous samples, were used to obtain a further check upon the relationship between the standard-quart and 16-ml techniques. This set numbered 134 samples, and the 16-ml determinations were made in quintuplicate, while the quart readings were taken but once as it was felt that the greater variability would exist in the micro method.

A third series of wheat was selected consisting of 140 samples from a wheat-breeding nursery. These samples were small in size and it was therefore impossible to carry out determinations by the standard method, which requires in excess of one quart of wheat. This series contained a great diversity of hard red spring types and was extremely variable in nature. Micro test-weight measurements were made with the 16-ml and 4-ml measures, in order to obtain further data on the correlation between the two procedures.

In the work at this Station the 16-ml method would be by far the most widely used of the micro methods. The 4-ml technique would only be resorted to in a limited number of cases where sufficient material was not available for the 16-ml method. This situation would arise at this Station only in plant nutrition studies.

Equipment Used for Micro Methods

In order to obtain comparable results with any method of micro weight determinations it was imperative to standardize strictly the equipment and procedure used. Glass graduated cylinders of 100- and 25-ml capacity were cut down to suitable size and the edges care-

fully ground smooth to contain accurately the desired quantity of wheat. The wheat was placed in a funnel made of heavy tin sufficiently large to hold an excess of wheat over that required to fill either measure. The funnel was fitted with a stand to hold it steady at a constant height of one inch above the center of the measuring cylinder. The lower opening of the funnel was $\frac{1}{2}$ inch in diameter and was equipped with a shutter to control the flow of the wheat. Preliminary tests with the 16-ml measure were conducted on two different types of stroker to determine which would be best suited for the micro method. One stroker was flat, $\frac{5}{8}$ inch in width and 7 inches long, with rounded edges. It closely resembled the one recommended in the official standard methods. The other stroker was round, with a diameter of $\frac{3}{8}$ inch and a length of 7 inches. Tests made with a small series of samples showed better agreement when the round stroker was used, and it was accordingly adopted for use in these investigations. A grain-testing balance with a sensitivity of 10 mg was used for weighing the measured wheat. The equipment used in conducting the micro tests is shown in Figure 1.

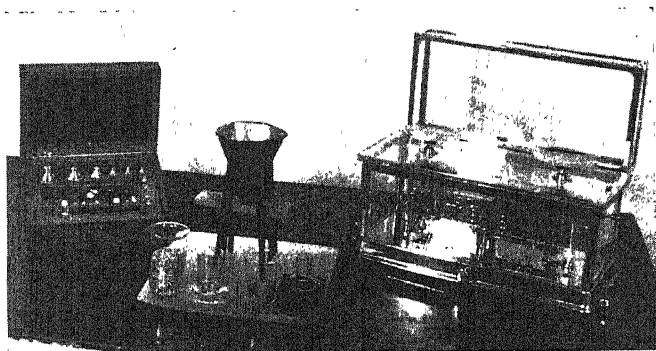


Fig 1. Equipment used in determining micro test weights. Funnel fitted with shutter and the 16- and 4-ml measures are shown in center, with the quantities of wheat required for each test. Weighing balance at right, stroker in front of measures.

Technique of Determination

The method used in the determinations was essentially the same for both the micro methods and has been described by Harris and Sibbitt (1941) in some detail. The wheat to be tested is placed in the funnel and the measure carefully positioned under its delivery end. The funnel shutter is then quickly opened and the entire quantity of grain allowed to run into the measure, filling it to overflowing. The stroker is then placed lightly on the edge of the measure, care being

taken not to jar or disturb the cylinder and its contents. The wheat is next leveled off with a zigzag motion of the stroker. The quantity of wheat remaining in the cylinder is then weighed.

The weight per 500 kernels was also determined in the first series, as this value has been considered to be related to kernel plumpness and test weight. It has been extensively used by agronomists and others interested in the quality of wheat.

As some difference had been experienced in connection with the packing of the kernels in the measure, especially when the 4-ml method was used, it was thought desirable to obtain information respecting the possible use of wheat meal as material for predicting test weight by micro methods. Air spaces between the kernels and the container would be eliminated, and a strictly level surface should be obtained following striking off. These factors would be more operative when the 4-ml method was employed. To settle this question a sufficient quantity of each sample in the first series was ground on a small Wiley mill to pass through a 20-mesh sieve, and micro test weights made on the resultant meal. As already pointed out a further set of tests was made on this series by two operators to determine whether there would be a significant difference in the two sets of data obtained occasioned by small variations in the procedure introduced by personal factors. The 16-ml micro method only was included in this study as this is the micro technique which is most useful in test-weight determinations in actual practice.

Discussion of Results

A scatter diagram of the 52 average results for the standard and 16-ml micro method is shown in Figure 2. It is clearly evident that a high positive correlation exists between the values yielded by the two methods. Similar diagrams are shown for the data from the standard and 4-ml micro method, as well as for the standard with the ground wheat meal by the 16-ml method in Figures 3 and 4, respectively. The relationship between the standard test weight and weight of 500 kernels is similarly represented in Figure 5. The regression line for predicting test weight (standard) from the weight in grams of material obtained by the various alternative micro methods is shown in these diagrams. The degree of correlation between the test weight and the other values shown decreases in the order named, with little relationship of importance apparent in Figure 5. The correlation coefficients obtained with corresponding fiducial limits, prediction equations, and standard errors of estimate are found in Table I.

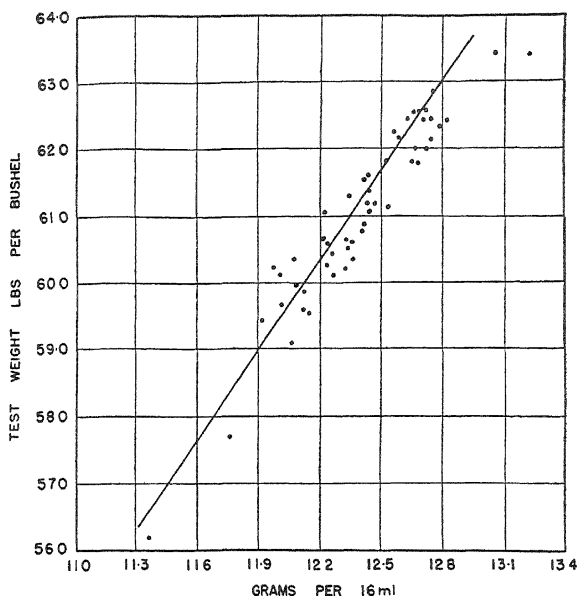


Fig. 2. Dot diagram of weight in grams of 16 ml of wheat and test weight per bushel.
 $N = 52$. $r = +.9539$.

TABLE I

CORRELATION COEFFICIENTS, WITH FIDUCIAL LIMITS, PREDICTION EQUATIONS AND ERROR OF ESTIMATES COMPUTED FROM DATA OF SERIES 1

Variables correlated		Correlation coefficients r_{xy}	Fiducial limits		Prediction equation	Standard error of estimate
X	Y		Lower	Upper		
Test weight, standard method, lbs	Micro 16-ml method, g	+0.9631	+0.9407	+0.9875	$4.56y + 4.7$	0.365
"	Micro 4-ml method, g	+0.8824	+0.7616	+0.9343	$21.05y - 0.05$	0.638
"	Micro 16-ml method, g (ground wheat meal)	+0.6951	+0.5169	+0.8155	$3.38y + 29.9$	0.977
"	Micro 4-ml method, g (ground wheat meal)	+0.7028	+0.5200	+0.8210	$13.24y + 31.0$	0.966
"	Weight 500 kernels, g	+0.5592	+0.4186	+0.7246	$0.38y + 56.1$	1.126

Fiducial limits represent the upper and lower limits according to a given probability level between which the true value of a statistic lies. In the present instance this probability level is the 5% point. These values are calculated from the distribution of z , rather than from that of the correlation coefficient r , and they are accordingly not subject to any uncertainty due to lack of normal distribution, as is the case for

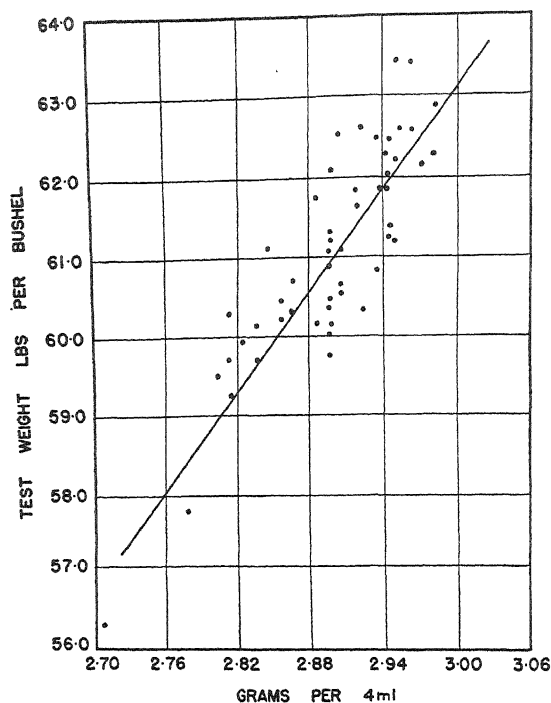


Fig. 3. Dot diagram of weight in grams of 4 ml of wheat and test weight per bushel.
 $N = 52$. $r = +.8332$.

certain values of the correlation coefficient, especially in the instance of small samples.

Table II shows the analyses of variance for the data obtained by

TABLE II
 ANALYSIS OF VARIANCE OF OPERATOR EFFECT IN MICRO TEST
 WEIGHT DETERMINATIONS

Source of variance	Sum of squares	Degrees of freedom	Variance	F	5% point	1% point
Between operators.....	0.151	1	0.151	37.8	3.89	6.76
Between samples.....	15.298	51	0.300	75.0	—	—
Interaction (operators × samples).....	10.532	51	0.203	50.8	—	—
Sampling error.....	0.806	208	0.004	—	—	—
Total.....	26.787	311	—	—	—	—

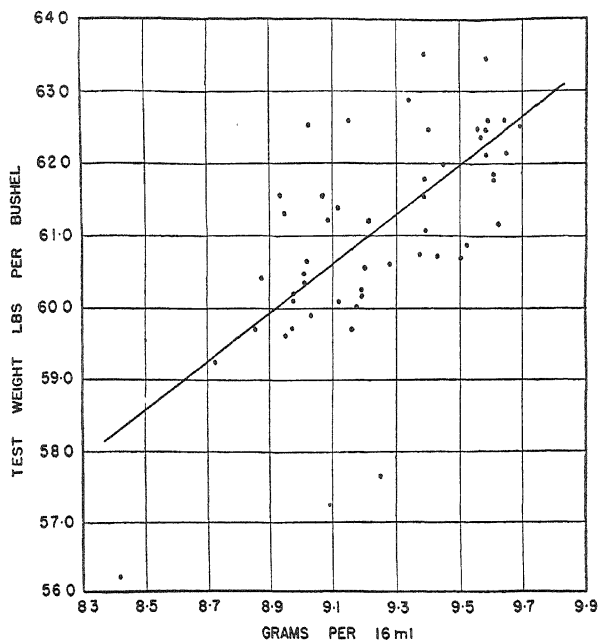


Fig. 4. Dot diagram of weight in grams of 16 ml of ground whole wheat meal and test weight per bushel. $N = 52$. $r = +.6933$.

the two different operators. As the F value computed is very much higher than the value at the 5% and 1% points the difference is clearly significant. This difference in results obtained by different operators is being thoroughly investigated at the present time, by four workers.

The second series of tests resulted in a correlation coefficient of $+0.9286$ between the standard-quart and the 16-ml micro methods, as shown in Table III. This statistic is in good agreement with the co-

TABLE III

CORRELATION COEFFICIENTS, WITH FIDUCIAL LIMITS, COMPUTED FROM THE DATA OF SERIES 2 ($N = 134$) AND SERIES 3 ($N = 140$)

Variables correlated		Correlation coefficients r_{zy}	Fiducial limits		Standard error of estimate
X	Y		Lower	Upper	
Test weight, standard method, lbs...	Micro 16-ml method, g	$+0.9286^1$	$+0.9011$	$+0.9486$	0.768
Micro 16-ml method, g...	Micro 4-ml method, g	$+0.9030$	$+0.8841$	$+0.9195$	—

¹ Prediction equation = $4.48y + 5.5$.

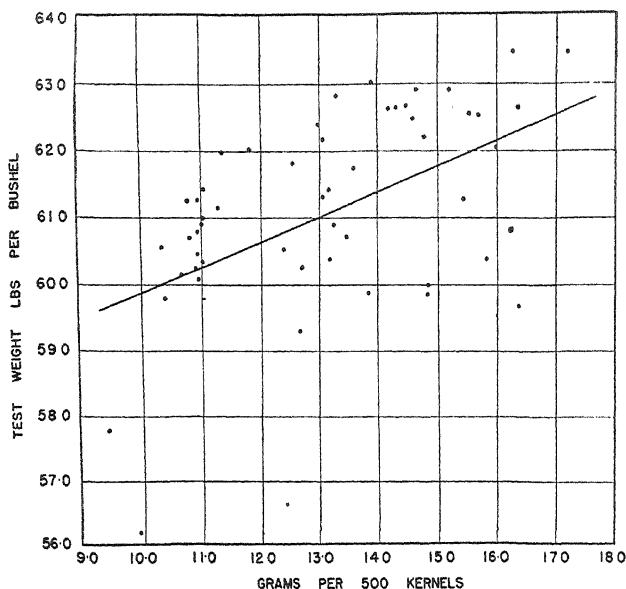


Fig. 5. Dot diagram of weight in grams of 500 kernels of wheat and test weight per bushel.
 $N = 52$. $r = +.5547$.

efficient found between the corresponding variables in the first series, and emphasizes the reliability of the test for the purpose of predicting test weight per bushel from small samples of wheat. The prediction equation, based on the two sets of data, yields results differing by not more than 0.2 lb in predicted weight per bushel within the usual range of test weights.

Table III contains also the statistical data from the third series, in which a comparison was made between the two micro methods. The results show that the weights obtained by the two methods are highly correlated, but as no attempt would probably ever be made to determine the weight in grams for the 16-ml method from the results of the 4-ml, no prediction equation is included in the data obtained from this series.

From the results described in this paper it is apparently possible to predict the weight per bushel as found by the usual official procedure from the weight in grams of 16 ml of the same wheat. It is also possible to predict, with somewhat less accuracy, the bushel weight from the weight in grams of 4 ml of the wheat. Ground wheat meal is not satisfactory as material for test-weight determinations, and the weight of 500 (or 1,000) kernels is quite unsatisfactory for prediction purposes.

Summary and Conclusions

Procedures have been described for the estimation of the test weight per bushel of hard red spring wheat samples too small to be tested by the official standard method. These methods include the weight in grams of: (1) 16 ml of wheat, (2) 4 ml of wheat, (3) 16 ml of finely ground wheat meal, (4) 4 ml of finely ground wheat meal, and (5) 500 kernels.

The 16-ml method gave the "most" satisfactory results, in respect to the degree of correlation with the standard method. The 4-ml method was the next best. Both these methods have distinct utility for the determination of test weight from small samples of wheat. The methods employing ground wheat meal were not highly correlated with test weight and would accordingly not be satisfactory for prediction purposes. The weight of 500 (or 1,000) kernels would be still less reliable for finding bushel weight.

Comparative determinations made by two operators on the same series of wheats with the 16-ml method showed significant differences between operators. This point is being further investigated. Suitable charts or tables can be constructed from which the standard test weight per bushel may be read off directly from micro results in grams. The findings confirm the usefulness of micro test-weight determinations as used at this Station.

Acknowledgments

The authors wish to acknowledge the assistance and advice of Clifford Maloney in the evaluation of the data. Thanks are also due to Works Projects Administration, Research and Records Division, through the operation of Seed Testing Project, O.P. 165-1-73-144, during this investigation, as well as to NYA funds for technical assistance. Mention should further be made of the Agricultural Marketing Service, U. S. Department of Agriculture, for their encouragement of the project, and to Dr. E. A. Helgeson, Head, Department of Botany, North Dakota State College, for his keen interest in the work.

Literature Cited

- Aamodt, O. S., and Torrie, J. H.
1934 A simple method for determining the relative weight per bushel of the grain from individual wheat plants. *Can. J. Research* 11: 589-593.
- Harris, R. H., and Sibbitt, L. D.
1941 An investigation of micro methods in comparison with the standard method of determining the test weight of hard red spring wheat. *N. Dak. Agr. Exp. Sta. Bimonthly Bul.* 3 (No. 4): 23-27.

A MICRO METHOD FOR DETERMINING TEST WEIGHT¹

C. O. SWANSON

Kansas Agricultural Experiment Station, Manhattan, Kansas

(Received for publication March 17, 1942)

Micro methods for the determination of test weight or pounds per bushel are needed in studies on wheat properties whenever the amount of grain available is insufficient for standard methods. Plant breeders need such methods because the amounts in available samples are small. Preharvest survey workers must use such methods because of limitations in procuring the larger samples. In estimating the relative ratios of kernel sizes in milling wheat in order to determine the relation of these sizes to probable flour yield and test weight, micro test weight methods will save much labor in making the separations.

There is but little information available on micro methods for determination of test weight. Aamodt and Torrie (1934) devised a method requiring 4-ml samples. The weight in grams of a 4-ml sample multiplied by 20 was found to give a close approximation to the test weight in pounds per bushel as determined by the usual apparatus. These investigators state that "The correlation coefficient calculated from the weight per bushel of 184 samples of spring wheat by these two methods was $+ .947 \pm .005$ and for 59 samples of winter wheat $+ .834 \pm .027$."

A study of micro test weight determination prepared by D. E. McCarty and J. W. Kirkbride is contained in a private communication from Dr. A. J. King, Agricultural Statistician of the Agricultural Marketing Service. Two brass cylinders of 16.08 ml and 32.5 ml capacity, respectively, were used. The test weights obtained by the micro method were somewhat smaller than the official method but they were sufficiently in agreement to warrant their use for research and some other purposes where the amount of material is insufficient to be used for the official method, Dr. King reported.

The need for a micro test weight method was revealed in the study of the effects of moisture on the physical properties of wheat (Swanson, 1941), because much labor could be saved by using small samples. A method was desired which would require only about 100 g of wheat. Three cups were made by cutting off the upper portions of cylindrical glass sample bottles and grinding a smooth edge. The cups were designated A, B, and C. The inside diameters of A and B were 5.4 cm, but A was 0.6 cm taller than B. The inside diameter of C was 4.4

¹ Contribution No. 80, Department of Milling Industry.

cm and the height was 1.9 cm taller than B. The volume capacities of the cups were: A, 106.5 ml; B, 91.5 ml; and C, 89.8 ml. Cup A held about 80 g, cup B about 69 g, and cup C about 68 g of wheat, depending on the test weight. It was planned that cup A should approximate the same ratio of depth to diameter as found in the official brass quart measure, and then have B with a lower height and C taller but with less diameter. This would give variations in capacities as well as ratios of diameters to depths to find which size agreed most nearly with the results of the official test weight method.

Formula for Calculation of Test Weight

The ratio between 35,238 ml in a bushel and 453.6 g in a pound is 77.69. Therefore, if a cup of the correct height and diameter had a volume of 77.69 ml, the weight in grams of wheat in this cup level full would be equal to the test weight in pounds per bushel. Since the cups used in this study had greater capacities, 77.69 divided by the capacities of the cups would give the factors by which to multiply the weight of wheat to obtain the test weights in pounds per bushel. These factors were: cup A, 0.73; cup B, 0.85; and cup C, 0.865. A table was calculated to permit rapid and direct readings.

Method of Procedure

A funnel through which the wheat was poured into the cup was obtained by cutting a glass funnel of 9.0-cm diameter a little above the juncture of the cone and stem and grinding to give an opening 1.4 cm in diameter. It is important that this opening be large enough to allow the wheat to flow freely but not too rapidly. The funnel is held in an ordinary ring stand and adjusted so that its opening is approximately two inches above the top of the cup. The cup is placed in a shallow pan to catch the overflow grain. The time for filling the cup should be such that the wheat kernels have somewhat the same freedom to flow and settle as in the official cup. In making a determination, the finger is held against the opening of the funnel so as to delay the flow of wheat until the funnel is full. The stroking or leveling of the wheat after the cup is filled to running over is made by a small ruler.

Statistical Analysis

With the method as outlined, the test weights of all the wheat samples used in the investigation mentioned (Swanson, 1941) were determined in duplicate for each of the cups A, B, and C. Since these wheat samples varied considerably in the official test weights they afforded a good basis to test the accuracy of the method.

Both official test weights and those obtained by cups A, B, and C were subjected to statistical analysis.² From this analysis of the test weights taken on the 95 samples by the official method and by the micro method in duplicate, using in turn cups A, B, and C, the following conclusions were reached:

1. The test weights obtained with cup A were significantly lower than the official.

2. The test weights obtained with cup B were slightly lower than the official, but the differences were not significant.

3. The test weights obtained with cup C were slightly higher than the official, but the differences were not significant.

4. The grand averages in pounds of the test weights of all the samples were: official 57.42; with cup B, 57.10; with cup A, 56.86; with cup C, 57.65.

5. The averages and standard errors were as follows: official, 57.42 ± 0.17 ; with cup A, 56.83 ± 0.17 ; with cup B, 57.09 ± 0.177 ; with cup C, 57.63 ± 0.200 .

The reasons for these variations were not investigated.

The study shows that a micro test weight method suitably designed is sufficiently accurate when only small samples of grain are available.

Literature Cited

Aamodt, O. S., and Torrie, J. H.

1934 A simple method for determining the relative weight per bushel of grain from individual wheat plants. *Can. J. of Res.* 11: 589-593.

Swanson, C. O.

1941 Effects of moisture on the physical and other properties of wheat. *Cereal Chem.* 18: 705-729.

² The writer is indebted to Mr. John A. Johnson, Jr., and Mr. William W. O'Donnell, Assistant and Research Assistant in the Department of Milling Industry, for making the statistical study.

THE EFFECT OF TEMPERATURE CHANGE DURING MALTING ON FOUR BARLEY VARIETIES ¹

H. L. SHANDS, ALLAN D. DICKSON, and JAMES G. DICKSON

University of Wisconsin and Division of Cereal Crops and Diseases, Bureau of
Plant Industry, U. S. Department of Agriculture, Madison, Wisconsin

(Read at Annual Meeting, May 1941)

In an earlier experiment with a small malting chamber, four barley varieties were malted at constant temperatures of 12°, 16°, 20°, and 24°C, respectively, for periods of two, four, six, and eight days, with two moistures, one relatively low and the other rather high. The results of this series were reported by Shands *et al.* (1941), who suggested that conditions which were changed systematically or periodically might more expressly influence malt quality. It also was realized that commercial malting is generally done under conditions where temperature is not held constant but changed at certain intervals. In view of these observations a series of 72 samples was malted in the small chamber, and temperature was changed at regularly scheduled time intervals. Under this set of conditions it was possible to observe the effects of several combinations using different time intervals at different temperatures and to make comparisons with malts produced under constant temperatures.

Plan of Experiment

The plan of the experiment here reported was to malt four barley varieties six days, using an intermediate moisture (45%) and the temperatures 12°, 16°, and 20°C singly and in varied sequence. Fifteen malting methods listed in Table I may be summarized as follows: three of the methods involved constant temperatures and 12 involved operations in which the above temperatures were shifted at time intervals of two or four days. For example, if a sample was malted at 12°C for two days, the temperature might be changed to either 16° or 20°C for the remaining four days. Or a sample might be malted for four days at 20°C and then changed to 16° or 12°C for the remaining two days. Duplicate maltings were made of the constant-temperature series, while single malts were produced in the temperature-change series.

¹ Based on cooperative investigations between the Wisconsin Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. The Federal WPA has contributed to the research program through a grant under the University of Wisconsin WPA Natural Science Project. The United States Maltsters Association has cooperated through an industrial fellowship grant to the University of Wisconsin.

TABLE I
PHYSICAL AND CHEMICAL ANALYSES OF MALT PRODUCED IN SMALL CHAMBER IN SIX DAYS AT CONSTANT TEMPERATURES
COMPARED WITH MALTS PRODUCED IN SIX DAYS WITH TIME AND TEMPERATURE CHANGED

Malting time and temperature														
Method no.	Steep and respi- ration %	Re-covery	Growth index	Kernel weight malt mg	Ex-tract, dry, malt %	Color	Dia- static power g/L	Time of con- version min	Malt in- crease %	Ratio of wort nitrogen to malt N %	Ratio of formal nitrogen to malt N %	Wet starch ratio g/100	pH	
A. ODERBRUCKER														
1	4.1	93.1	81.3	28.6	75.5	1.2	170	7.3	1.99	36.2	14.2	6.1	6.1	
2	5.0	90.3	85.0	28.5	74.6	1.4	177	<5	1.98	34.4	13.6	6.0	6.0	
3	6.6	88.6	86.9	27.7	73.8	1.4	172	6.8	2.00	35.2	12.0	5.9	5.9	
4	3.9	92.4	78.5	28.9	74.7	1.3	174	5-7	1.99	34.3	13.4	6.0	6.0	
5	4.5	91.4	78.0	28.4	74.9	1.3	166	5-7	1.97	33.9	13.9	6.1	6.1	
6	4.9	91.3	80.3	28.5	75.0	1.5	172	5-7	1.97	34.0	12.6	5.9	5.9	
7	6.2	89.0	86.8	28.0	73.6	1.6	175	<5	1.96	32.9	12.1	5.5	5.5	
8	4.6	92.2	86.0	28.8	75.3	1.5	186	<5	1.98	37.1	15.2	6.0	6.0	
9	4.3	92.5	78.5	28.9	75.5	1.5	185	<5	1.98	37.1	15.0	5.7	5.7	
10	5.8	89.1	82.5	28.4	74.5	1.3	180	5-7	2.00	33.6	11.9	6.0	6.0	
11	5.9	89.0	78.3	28.1	74.0	1.4	174	5-7	1.99	32.0	12.2	5.8	5.8	
12	6.0	89.6	90.3	28.5	74.8	1.4	170	7-10	1.97	34.8	13.5	6.1	6.1	
13	5.5	91.1	99.0	28.3	75.3	1.4	180	7	1.91	40.0	16.1	6.2	6.2	
14	6.4	89.8	84.5	27.8	74.2	1.6	177	<5	2.00	32.0	12.3	6.0	6.0	
15	5.1	91.4	89.5	28.2	74.4	1.5	171	<5	1.99	36.1	13.9	6.1	6.1	
B. WISCONSIN BARBLESS														
1	3.6	93.8	86.2	29.3	73.6	1.3	114	10-15	1.96	29.3	11.2	6.1	6.1	
2	4.7	91.4	85.0	28.4	73.3	1.3	115	10	1.93	29.3	11.3	6.0	6.0	
3	5.8	89.3	84.2	27.9	72.6	1.4	124	10-15	1.95	31.8	9.8	5.9	5.9	
4	3.1	93.7	72.3	28.6	73.0	1.1	109	10-15	1.92	28.0	10.9	6.0	6.0	
5	3.7	92.6	72.0	28.6	73.2	1.3	113	10-15	1.93	26.2	11.3	6.2	6.2	
6	4.3	92.1	78.5	28.8	73.2	1.1	120	10-15	1.94	28.0	10.2	6.0	6.0	
7	5.5	90.0	85.0	27.8	72.4	1.6	120	10	1.98	25.8	9.1	5.6	5.6	
8	4.1	93.0	84.5	28.7	73.4	1.5	127	10	1.93	31.4	12.4	5.8	5.8	
9	3.6	93.3	78.8	29.1	73.5	1.4	123	7-10	2.00	29.6	11.7	5.7	5.7	
10	5.0	90.7	79.8	28.1	73.3	1.1	121	10-15	1.92	28.1	10.5	6.0	6.0	
11	4.9	90.7	74.3	28.9	72.6	1.3	114	10-15	1.90	26.6	10.2	5.8	5.8	
12	4.8	90.9	83.3	28.3	73.4	1.1	116	15	1.92	28.4	10.9	6.1	6.1	
13	5.1	91.7	96.3	28.5	74.1	1.1	124	10-15	2.03	31.3	12.1	6.2	6.2	
14	5.9	90.4	85.5	28.0	73.2	1.4	123	10	1.96	27.2	10.1	6.0	6.0	
15	5.0	91.1	93.0	28.7	73.1	1.4	121	7	1.90	34.5	11.9	6.2	6.2	

¹ Average of duplicate maltings.

TABLE I (Continued)

Malting time and temperature	Method no.	Steep and respiration loss	Recovery	Growth index	Kernel weight	Extract, dry basis	Color	Dia-static power	Time of conversion	Malt nitrogen	Ratio of wort nitrogen to malt N	Ratio of formal nitrogen to malt N	Wort acidity
		%	%	no.	mg	%	Low β ₂	°L	min	%	%	%	pH
C. PEATLAND													
6 days at 12° C (constant) ¹	1	4.1	92.9	81.7	21.8	74.4	1.4	159	7-10	2.09	36.2	14.0	6.1
6 days at 16° C (constant) ¹	2	5.4	90.5	81.3	20.8	74.1	1.6	172	7	2.08	38.0	14.9	6.1
6 days at 20° C (constant) ¹	3	7.2	87.8	86.1	20.6	72.8	1.8	156	6.3	2.14	36.2	12.8	5.9
4 days at 12°, 2 days at 16° C	4	3.8	92.7	74.5	21.3	74.3	1.5	153	5-7	2.06	37.1	14.6	5.9
2 days at 12°, 4 days at 16° C	5	4.5	91.2	72.8	21.5	74.0	1.6	156	7-10	2.06	37.9	15.1	6.2
4 days at 12°, 2 days at 20° C	6	5.5	90.1	79.8	21.5	73.9	1.5	162	7-10	2.08	36.3	13.8	6.0
2 days at 12°, 4 days at 20° C	7	6.7	88.0	90.0	21.0	72.7	1.9	157	<5	2.12	34.8	13.0	5.8
4 days at 16°, 2 days at 12° C	8	4.7	92.3	85.8	21.8	74.3	1.6	174	5	2.09	38.2	15.0	5.8
2 days at 16°, 4 days at 12° C	9	4.4	92.3	82.0	21.0	74.8	1.5	177	<5	2.09	39.8	16.0	5.8
4 days at 16°, 2 days at 20° C	10	6.2	88.9	80.0	20.9	73.5	1.4	158	7	2.10	34.6	13.2	6.0
2 days at 16°, 4 days at 20° C	11	6.5	88.5	74.8	20.4	73.5	1.8	150	7-10	2.14	33.4	13.0	5.8
4 days at 20°, 2 days at 12° C	12	6.2	89.2	89.5	20.6	73.8	1.4	145	10	2.11	36.0	14.1	6.1
2 days at 20°, 4 days at 12° C	13	6.2	90.4	97.3	21.3	74.3	1.5	172	10	2.17	37.4	15.2	6.2
4 days at 20°, 2 days at 16° C	14	7.1	88.8	84.3	20.8	73.5	1.9	155	5	2.10	35.6	13.6	6.1
2 days at 20°, 4 days at 16° C	15	5.8	90.3	87.3	21.1	73.5	1.9	153	5	2.18	36.7	14.0	6.2
D. CHEVRON													
6 days at 12° C (constant) ¹	1	4.1	93.1	79.4	22.3	74.0	1.4	208	5.5	2.27	36.9	14.2	6.1
6 days at 16° C (constant) ¹	2	5.6	90.0	79.7	20.8	73.4	1.6	224	<5	2.27	35.9	13.9	6.0
6 days at 20° C (constant) ¹	3	7.3	87.3	82.8	20.7	72.3	1.7	206	6.3	2.31	35.7	12.0	5.8
4 days at 12°, 2 days at 16° C	4	3.7	92.5	74.0	22.0	74.5	1.5	201	5-7	2.24	36.2	14.1	5.8
2 days at 12°, 4 days at 16° C	5	4.6	90.4	71.5	21.3	73.6	1.5	215	5	2.26	35.2	14.2	6.1
4 days at 12°, 2 days at 20° C	6	5.4	90.3	78.8	21.6	73.6	1.4	207	5-7	2.25	34.7	12.8	6.0
2 days at 12°, 4 days at 20° C	7	6.7	88.1	87.5	21.1	71.9	1.6	201	<5	2.27	34.0	11.8	5.7
4 days at 16°, 2 days at 12° C	8	4.8	91.7	80.5	21.7	74.0	1.8	239	<5	2.29	36.3	14.1	5.8
2 days at 16°, 4 days at 12° C	9	4.4	92.2	74.3	21.7	74.0	1.6	230	<5	2.24	38.7	15.0	5.6
4 days at 16°, 2 days at 20° C	10	5.7	88.9	76.8	21.2	72.7	1.4	202	5-7	2.28	33.7	12.2	5.7
2 days at 16°, 4 days at 20° C	11	6.3	88.5	76.5	21.2	72.8	1.6	199	7	2.28	33.0	12.2	5.8
4 days at 20°, 2 days at 12° C	12	5.9	89.8	79.3	21.4	73.0	1.5	186	10	2.31	33.2	12.7	6.1
2 days at 20°, 4 days at 12° C	13	6.2	90.2	96.8	21.2	73.6	1.5	218	5-7	2.31	36.7	14.4	6.2
4 days at 20°, 2 days at 16° C	14	6.8	89.3	78.5	21.2	72.7	1.6	210	5	2.31	33.5	12.5	6.1
2 days at 20°, 4 days at 16° C	15	5.9	89.9	87.8	21.5	72.9	1.8	200	<5	2.38	34.0	12.7	6.0

¹ Average of duplicate maltings.

Materials and Methods

The four barley varieties used were grown in 1939 and were the same as those for the experiment reported by Shands *et al.* (1941), namely (1) Oderbrucker (Wisconsin Pedigree 5-1), (2) Wisconsin Barbless (Wisconsin Pedigree 38), (3) Peatland, and (4) Chevron. Lots of the first two varieties came from Wisconsin farmers' fields in regular agricultural production. Small kernels were removed from these lots by cleaning and sizing equipment. Peatland and Chevron were grown in the yield trial plots on the West Hill Farm of the Wisconsin Agricultural Experiment Station. After threshing, these latter two varieties were subjected to cleaning by means of a Clipper mill. The barleys had the following kernel weights in milligrams, dry basis: 31.7, 31.2, 22.9, and 24.0, respectively, for the varieties as listed above. The barley nitrogen percentages in the same order were as follows: 2.02, 1.96, 2.06, and 2.24.

All samples were malted six days in a modified General Electric refrigerator equipped to provide positive circulation of humidified air and relatively accurate temperature control. Small screen-bottomed cans were used for steeping, germinating, and drying as described previously (1941). The same kiln or drier used in the earlier experiment was adjusted to dry the malts to approximately 5% moisture during a period of 31 hours. Thermostatically controlled electric heaters were used for the drying process.

A sample of each variety of barley was subdivided into 18 portions, each weighing 170 g, dry basis. Samples were placed into the steep tank at time intervals appropriate for absorbing 45% moisture at 16° C. The amounts of time required for steeping were 41, 43, 33, and 33 hours respectively, for the four barley varieties. The growing malts were stirred twice daily after being watered. The watering schedule was based on the expected respiration losses. The moisture content of the green malt, averaging slightly above 44%, indicated that the watering schedule was satisfactory.

Physical and Chemical Analysis

The 72 malts were analyzed for the following physical and chemical factors: steep and respiration loss, recovery, growth of acrospire, kernel weight, extract, diastatic power, conversion time, malt nitrogen, wort nitrogen, formol nitrogen, and pH of wort. The ratio of wort and formol nitrogen to malt nitrogen were calculated. Other factors studied, the results of which are not given in Table I, are: moisture content of steeped barley, green and dried malt, cleaning loss, and permanently soluble nitrogen. The analyses were conducted as described by Dickson and Burkhart (1942), and Shands *et al.* (1941).

Steep and Respiration Loss

Steep and respiration losses given in detail in the four sections of Table I ranged with the barley variety or malting method used, from as low as 3.1% to as high as 7.3%. Losses for Peatland and Chevron were greater than for Oderbrucker, those for Wisconsin Barbless being lowest. Averaging the four varieties, Method 4 (four days at 12° followed by two days at 16°C) gave the lowest loss, and the constant-temperature method of six days at 12°C gave a loss only slightly higher. The highest loss occurred at the constant temperature of 20°C. The results as averages for the four varieties are summarized in Table II and Figure 1A.

TABLE II

SUMMARY OF EFFECT OF DIFFERENT MALTING METHODS ON QUALITY FACTORS
—AVERAGE OF FOUR VARIETIES

Malt quality factor	Malting time (days) at various temperatures, and method number														
	6 at 12°	6 at 16°	6 at 20°	4 at 12° 2 at 16°	2 at 12° 4 at 16°	4 at 12° 2 at 20°	2 at 12° 4 at 20°	4 at 16° 2 at 12°	2 at 16° 4 at 20°	4 at 16° 2 at 20°	2 at 16° 4 at 20°	4 at 20° 2 at 12°	2 at 20° 4 at 12°	4 at 20° 2 at 16°	2 at 20° 4 at 16°
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Steep and respiration loss, %	3.9	5.2	6.7	3.6	4.3	5.0	6.3	4.6	4.2	5.7	5.9	5.7	5.8	6.6	5.5
Recovery, %	93.2	90.5	88.2	92.8	91.4	91.0	88.8	92.3	92.6	89.4	89.2	89.9	90.9	89.6	90.7
Extract, dry basis, %	74.3	73.8	72.9	74.1	73.9	73.9	72.7	74.3	74.5	73.5	73.2	73.8	74.3	73.4	73.5
Color, Lov	1.3	1.5	1.6	1.3	1.4	1.4	1.7	1.6	1.5	1.3	1.5	1.4	1.4	1.6	1.6
Diastase, °L	162	172	164	159	163	165	163	182	179	165	159	154	173	166	169
Conversion time, min	83	64	75	7.6	8.0	8.5	5.5	5.8	5.0	7.9	8.5	10.9	8.9	5.8	4.8
Ratio of wort N to malt N, %	34.6	34.4	34.7	33.9	33.3	33.3	31.9	35.8	36.3	32.5	31.5	33.1	36.4	32.1	35.3
Ratio of formol N to malt N, %	13.4	13.4	11.6	13.4	13.6	12.4	11.5	14.2	14.4	12.0	11.9	12.8	14.5	12.1	13.1

Recovery

Recovery ranging from 87.3% to 93.8% was influenced by variety and method of malting. For Chevron and Peatland it was similar, being slightly less than Oderbrucker, which in turn was less than Wisconsin Barbless. Peatland and Chevron barleys were grown under field conditions different from those of Oderbrucker and Wisconsin Barbless, and for this reason indiscriminate varietal comparisons for recovery or other factors may be misleading. Averaging of values showed lowest recovery, 88.2, to result from the constant temperature of 20°C and highest, 93.2, from the constant temperature of 12°C. The next high recovery was with four days at 12° followed by two days at 16°C.

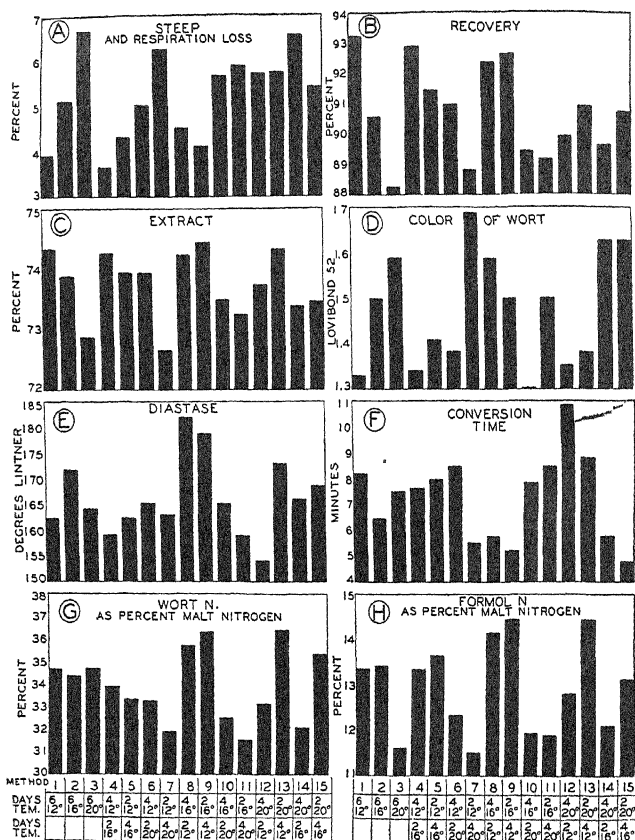


Fig. 1. Summary of the effect of different malting methods on quality factors.

Growth of Acrospire

Growth index of acrospire ranged from 71.5 to 99, being influenced by both barley variety and malting method. Method 13, with two days at 20° and four days at 12°C, gave the highest growth index. Why this method gave more growth than occurred with other methods is not understood.

Kernel Weight

Kernel weights varied slightly around two sets of means, one for Oderbrucker and Wisconsin Barbless, and another for Peatland and

Chevron. The method giving highest kernel weight involved, as in the case of recovery, the constant 12°C and, similarly, the lowest involved the constant 20°C. The methods involving change of time and temperature gave intermediate values.

Extract

Malt extract values varied over a fairly narrow range of 71.9 to 75.5. Oderbrucker extract was about 1½% greater than Wisconsin Barbless, while Chevron was about ½% lower than Peatland. Method 7, two days at 12° and four days at 20°C, gave the lowest average extract percentage, followed closely by the constant 20°C. Highest extract was obtained by Method 9, which was two days at 16° and four days at 12°C. This was followed closely by other methods as can be seen in Table II and Figure 1C.

Color

Wort color readings were low for the most part, varying from 1.1 to 1.9 (Lov. 52). Peatland and Chevron gave higher colored worts than Wisconsin Barbless and Oderbrucker. Method of malting also influenced color over a range of 1.3 to 1.7, as will be seen in Figure 1D.

Diastatic Power

Enzyme development as measured by diastatic power or conversion time was greatly influenced by both barley variety and malting methods. The range in degrees Lintner was from 109 for Wisconsin Barbless to 239 for Chevron. The largest differences were attributable to varietal response. Oderbrucker was higher in diastatic power than Wisconsin Barbless, and Chevron was higher than Peatland, comparisons which agree with previous studies. The method responsible for the highest average diastase value was No. 8, or four days at 16° and two days at 12°C. Table II and Figure 1E show that Method 8 was followed closely by No. 9; and the lowest diastase was produced by Method 12, or four days at 20°C and two days at 12°C.

Conversion Time

Several of the malts showed a conversion time of less than 5 minutes while one was 15 minutes. Wisconsin Barbless was slower in conversion time than the other varieties, and Peatland seemed slower than Chevron. In deriving average figures for conversion time in Table II, less than 5 minutes was arbitrarily calculated as 4 minutes, and 10–15 minutes was given the value of 12.5. There seems to be an association of high diastase and low conversion time, though by no means a perfect correlation. When conversion time was averaged for the four

varieties, Method 15, two days at 20° and four days at 16°C, showed the shortest time for conversion. The results are given graphically in Figure 1F.

Malt Nitrogen

Malt nitrogen appeared to be largely a varietal characteristic. Variation in amount caused by different methods was small and of doubtful significance. Wisconsin Barbless averaged slightly lower in nitrogen than Oderbrucker, a result which could be expected on the basis of the nitrogen content of the original barley. Chevron malts were higher in nitrogen than Peatland malts.

Wort Nitrogen

The ratio of wort nitrogen to malt nitrogen expressed as a percentage varied considerably. The range was from 25.8% to 40.0%, depending upon variety and malting method. Peatland had a higher wort nitrogen ratio than Chevron, and Oderbrucker was higher than Wisconsin Barbless. When varieties were averaged, the method of two days at 20° and four days at 12°C gave the highest ratio, while two days at 16° and four days at 20°C gave the lowest ratio. Method 9 also gave a high ratio of wort to malt nitrogen.

Formol Nitrogen

Formol nitrogen seemed to be associated with wort nitrogen. There was a range from 9.1% to 16.1%, depending upon variety and malting method. As in the case of the wort nitrogen ratio, two days at 20° and four days at 12°C gave the highest formol nitrogen ratio. Lowest formol nitrogen ratio was obtained in the reciprocal method, namely two days at 12° and four days at 20°C.

pH Reaction of Wort

The pH of the worts varied from 5.5 to 6.2. Varieties reacted similarly for the most part, while methods produced rather definite differences. Two days at 12° and four at 20°C, the method that gave lowest formol and second low wort nitrogen ratios, gave the lowest pH value. The high pH value was obtained with two days at 20° and four days at 12°C, which was the method giving highest formol and wort nitrogen ratios.

Summary and Discussion

Summaries of several of the malting factors used in this study are given in Table II and Figure 1. The values listed are averages of the four varieties for a particular method of malting. The data given in

Table I have been subjected to statistical treatment, and statements made in this paper have the benefit of this analysis. In interpretation and application of the results, several conditions must be kept in mind. First, the limiting of all methods to six days of malting necessarily prevented daily interval observations for sequence of chemical and physical changes. Second, all methods were limited to a single moisture during growth. It is therefore impossible to show the compensating effect of different moistures. In other words the experiments determined the combined influence of temperature change for the six-day malting period and do not show the progressive modification and chemical composition at different stages or intervals of growth. Only a six-day cross section of the malting process was available, and what would be applicable to six-day malts might not apply to four-, five-, or seven-day malts. In an earlier experiment where malts were grown for two-, four-, six-, and eight-day intervals, trends for various factors were available for interpreting the data.

Malting methods which gave low steep and respiration losses gave high values for recovery, extract, and formol nitrogen. Recovery percentages also closely paralleled those of extract and formol nitrogen, and less closely those of wort nitrogen. High extract was associated with high formol nitrogen ratios, and to a less extent with a high wort nitrogen ratio. There was a suggestion that high extract values and prolonged conversion times were related to low wort colors. More diastatic power usually accompanied higher wort and formol nitrogen ratios and shorter conversion time. Wort and formol nitrogen ratios responded somewhat similarly to different malting conditions. Restating some of these relationships, formol nitrogen was positively correlated with extract, recovery, wort nitrogen, and diastatic power, and negatively correlated with steep and respiration losses. Wort nitrogen ratios followed similar trends with diastase, extract, and recovery. Thus it will be seen that several important interrelationships existed in this group of malts produced under the limited conditions already described.

The use of different steep and malting moistures might serve to bring out malting responses that would otherwise be overlooked. With emphasis on caution in interpretation or application of the results described in this paper, a few methods either of constant or changed conditions may be compared. Methods 8 and 9 under experimental conditions produced malts that were apparently superior to other malts produced either by constant or changed conditions. Malts produced by using two days at 16° and four days at 12°C were high in recovery, extract, diastase, ratios of wort and formol nitrogen, low in steep and respiration loss, and low in conversion time, with color being accept-

able. In contrast, two days at 16° and four days at 20°C gave malts low in recovery, extract, diastase, ratios of wort and formol nitrogen, and high in steep and respiration loss, and high in conversion time, with an intermediate color. These results indicate that within the ranges used, reduction in temperature after germination has progressed for a short time might improve quality, and conversely a temperature rise after germination is well started might deplete quality.

The fact that inferior malt was produced by a particular method does not necessarily imply that all of its features are bad. By reducing growth time, for example from six to five days at 20°C, a more desirable malt from the standpoint of recovery and extract may be produced. Other modifications in malting method may likewise improve quality. Furthermore length of malting time might be of considerable importance in plant operation. If less than six days had been used, the above methods might have produced different relationships.

The results of this experiment, in general, point to the possibility of improving malt quality by relatively simple temperature change during the malting period. They also indicate the desirability of further experimentation in finding responses to additional changes that could be made in malting. If temperatures were gradually reduced or increased, malting factors might be expressed somewhat differently than under conditions of constant temperature. Moisture change during malting might also influence the expression of quality factors. Using different moisture levels out of steep in combination with different growth periods and different temperature schedules might offer approaches for the production of more desirable quality and at the same time maintain efficient plant operation. Since previous experimentation with the physiology of malting has netted sizable returns in improving quality, it seems logical that further experiments along the lines suggested might open avenues for still more improvement.

Acknowledgments

The writers acknowledge the assistance of L. A. Hunt and W. W. Pregler in the operation of the small malting chamber in this experimental series. B. A. Burkhart and J. H. Torrie aided in the chemical analysis of the malts and statistical analysis of data, respectively.

Literature Cited

- Dickson, A. D., and Burkhart, B. A.
1942 Changes in the barley during malting; chemical comparisons of germ and distal portions. *Cereal Chem.* 19: 251-262.
Shands, H. L., Dickson, A. D., Dickson, J. G., and Burkhart, B. A.
1941 The influence of temperature, moisture and growth time on the malting quality of four barley varieties. *Cereal Chem.* 18: 370-394.

EFFECTIVENESS OF DRY MILK SOLIDS¹ IN PREVENTING OVERBROMATION OF SOME BLEACHED HARD WINTER WHEAT FLOURS²

GLENN A. WEST³ and E. G. BAYFIELD⁴

Kansas State College, Manhattan, Kansas

(Received for publication December 19, 1941)

In recent years considerable work has been done to show the buffering effect of dry milk solids towards excessive amounts of potassium bromate in the bread formula. Various investigators have reported somewhat similar dough-improving effects from the use of bleaching agents and from potassium bromate. Since it is a common practice to use various oxidizing agents when baking bleached flours, the following question has been raised: Will the use of dry milk solids prevent the deleterious effects resulting from a combination of excessive quantities of potassium bromate and bleaching agents? The present investigations were undertaken to study this problem.

Review of Literature

The present common practice of supplementing the baking formula with milk is relatively new to the commercial baker, although an ancient practice of the housewife. Since Sherman, Rouse, Allen, and Woods (1921) investigated milk in bread from a nutritive viewpoint there has been considerable attention given to the study of milk, particularly dry milk solids, in bread.

Studying the action of phosphatides in dough, Working (1928a) found that if 4% to 6% of dry milk solids were added to the bread formula an improvement could be obtained by the addition of both a phosphatide and an oxidizing agent.

St. John and Bailey (1929) demonstrated that the buffering action of dry milk solids was appreciable, as shown both by the initial hydrogen-ion concentration of the freshly mixed doughs and by the relative rate of change in pH of control and milk-containing doughs. They concluded that the greater stability of milk doughs might be the consequence of the slower change in hydrogen-ion concentration. Skovholt and Bailey (1932) observed greater improvements by adding malt to doughs containing dry milk solids than to milk-free doughs. Increased scores resulted from the use of Arkady in every case when

¹ The term "dry milk solids" refers to the product made by removal of the water and fat from milk and contains not over 1 1/2% fat and not over 5% moisture.

² Contribution No. 78, Department of Milling Industry. Condensed from a thesis submitted to the graduate faculty, Kansas State College, in partial fulfillment of the requirements for the degree of Master of Science.

³ Research Assistant, American Dry Milk Institute, Chicago, Illinois, at Kansas State College.

⁴ Head, Department of Milling Industry, Kansas State College.

used in doughs containing dry milk solids. The improvement obtained by the use of Arkady under these conditions was much greater with winter wheat flours than with spring wheat flours.

Larmour, Working, and Ofelt (1939), using hard winter wheat flours, observed that the addition of 6% dry milk solids to the bread formula made it possible to include sufficient potassium bromate to condition the flours of the highest bromate requirement without over-oxidizing those of very much lower requirement. Using 4% dry milk solids, Barmore, Finney, and McCluggage (1939) report the beneficial effect of milk and bromate when included in the baking formula. These studies were expanded to include additional crop season samples and have been reported by Finney and Barmore (1941). Ofelt and Larmour (1940) observed that dry milk solids, together with the appropriate amounts of bromate, produced increases in loaf volume and improvements in texture beyond what could have been obtained with optimum amounts of potassium bromate alone. They pointed out the commercial significance of the buffering effect of dry milk solids toward bromate as being a safeguard against the possibility of damaging flours that had already been brought close to their optimum "oxidation" condition by bleaching or by addition of other oxidizing agents. This work was confirmed by Eisenberg (1940) and by Harris and Bayfield (1941), who presented corroborative data.

Working (1928b) observed that bleaching of flour had the same or a similar effect in developing the dough made from that flour as the addition of oxidizing agents to the dough. Hanson (1932) found that double the amount of nitrogen trichloride required to effect a full bleach did not in any way impair the baking quality of the flour. He observed that the addition of potassium bromate to the full bleach and to the double-bleach treatments did not show any beneficial results.

Rich (1934) observed that the action of potassium bromate appeared to be similar to that of nitrogen trichloride but more pronounced. He suggested that the maturing effect of bromate and nitrogen trichloride seemed to be dependent on the protein content of the flour. He indicated that the maturing action of nitrogen trichloride depended primarily on some reaction with the germ content of the flour. The similar effect of bromate suggested the reaction to be on of oxidation.

Balls and Hale (1936) suggested that the alteration of flours by bleaching was due to a diminution of the proteolytic activity, brought about by the oxidation of the activator of the flour proteases.

Harris and Bayfield (1941) found that bread was improved by the addition of increasing amounts of Agene and Novadelox, but that it was impossible to equal the improvements produced by the addition of bromate. Because of these differences they concluded that "bro-

mate action" and "bleaching action" should not be used interchangeably as meaning the same thing.

Working (1928b) attributed the beneficial effects of small amounts of oxidizing agents to their action in rendering phosphatides more soluble in water. Geddes (1930) concluded that the response of a flour to bromate depended on the state of oxidation of the germ constituents. Geddes and Larmour (1933) found that as the quantity of bleach was increased there was a definite trend towards decreased bromate response.

Rich (1934) suggested that the action of flour improvers must be colloidal, as the small amounts used could not effect gross chemical changes. His investigation indicated that bromate acted on the germ and protein constituents. Jørgensen (1936) restated his arguments in which he discredited the phosphatide and "electrolyte" theories and suggested instead that bromate paralyzes the proteinases, or their activators, of the wheat flour.

It was suggested by Sullivan, Howe, and Schmalz (1936) that the water-soluble substance responsible for the deleterious effects of germ was glutathione. They suggested that oxidizing agents changed some of the S-H glutathione to the S-S form which was unable to activate the proteases.

Mohs (1940) attributed the effect of flour improvers to their influence on the physical phase of the flour constituents. He suggested that their main influence was concerned with changes of surface tension and consequently the regulation of swelling processes or enzyme activity.

It is evident from a study of the literature that no majority agreement has been reached as to the fundamental reason for the improvement in bread which results from the use of commercial bleaching agents, potassium bromate, and milk. It is also evident that the use of milk in bread-making improves the product and guards against failure resulting from accidental overbleaching of the flour or overactivity of the dough.

Materials and Methods

The wheat varieties selected for this investigation were Tenmarq, Turkey (Kansas No. 570), Chiefkan, and Nebred. As a result of growing conditions, the samples were higher in protein content than is normally desired for bakery flour production. This made test baking difficult, especially when using the milk formula which produced large volumes that were somewhat hard to replicate satisfactorily. Table I gives analytical data for these samples.

With the exception of Chiefkan, these wheats were pure varieties. Chiefkan, however, was known to contain approximately 15% of other

TABLE I
ANALYTICAL DATA FOR WHEAT AND FLOUR

Variety	Test weight	Wheat		Flour			
		Protein ¹	Ash ¹	Yield ²	Protein ¹	Ash ¹	Absorption ^{1,3}
	<i>lbs</i>	%	%	%	%	%	%
Tenmarq	57.8	16.3	1.557	71.6	15.7	0.372	65.4
Turkey	59.2	15.4	1.621	70.1	14.5	0.384	61.5
Nebred	60.7	15.4	1.585	72.7	14.8	0.397	61.1
Chiefkan	60.6	16.5	1.430	71.7	15.8	0.379	56.7

¹ Moisture basis 15%.

² Total weight of flour on basis of dry cleaned wheat.

³ Values for milk-free formula without bromate.

winter wheats of good baking quality, and was materially benefited by this admixture. The extent of the improvement brought about by the impurity in the Chiefkan is indicated in Table II.

TABLE II
THE EFFECT OF THE 15% IMPURITY GROWN WITH CHIEFKAN¹

Sample	Wheat protein ²	Loaf volume
	%	cc
Pure Chiefkan	15.6	830
Bearded wheat impurity	16.7	1250
Mixture	15.9	950

¹ The separation of Chiefkan from other wheats was made from bundles of unthreshed grain.

² Moisture basis 15%.

Ten-bushel samples of Tenmarq, Turkey, and Chiefkan were milled on the Kansas State College 65-barrel mill. These three wheats were grown under comparable conditions at Manhattan. Only a limited supply of Nebred was available; therefore it was milled on a Buhler experimental mill. The Nebred sample was made up from two lots of this variety, the larger proportions being grown at the Nebraska Agricultural Experiment Station, Lincoln, Nebraska. The experimental flours were placed in a refrigerated room (40°-45°F) immediately after milling to reduce natural aging and bleaching to a minimum.

The baking formula used in this investigation was identical with that used by Harris and Bayfield (1941), as follows:

	<i>Grams</i>
Flour...	100.00
Sugar.....	6.00
Salt.....	1.50
Yeast.....	2.00
Shortening.....	3.00
Malt syrup (120° L).....	0.25
Water (distilled).....	As required
Dry milk solids.....	(0 or 6) as indicated
Potassium bromate.....	(0 to 0.007) as indicated

The addition of 6% of dry milk solids resulted in an increase of 4% in the absorption value. The absorption for each variety was held constant without regard to the bleaching treatment, although the absorption was increased with increasing increments of potassium bromate.

Doughs were mixed at 100 rpm in a Swanson-Working mixer, using a bowl containing two opposite pins. The doughs were all given an optimum mixing time, and were fermented and proofed at 86°F and 80% relative humidity.

Fermentation was as follows:

	<i>Minutes</i>
First punch after	105
Second punch after	50
Mold after additional	25
Total	180

A National "pup" sheeting roll was used for punching while the doughs were molded on a Thompson laboratory molder. The loaves were baked at 420°F for 25 minutes in a specially designed Despatch oven equipped with a rotating hearth. Loaf volumes were taken immediately upon removal of the loaves from the oven. All bakes were replicated on different days for loaf volume to agreement within 25 cc. The loaves were cut and graded for internal characteristics approximately 16 hours later. The baking laboratory was conditioned, the temperature being held between 76° and 78°F.

Agene was added to the flour in quantities equivalent to 3 g and 9 g per barrel, which is referred to as normal bleach and 3-normal bleach, respectively. Novadelox was added to the flour in quantities equivalent to 0.4 oz and 1.2 oz per barrel, which is also referred to as normal and 3-normal bleach. All bleached samples were stored for two weeks before baking in tightly closed cans in a laboratory held at 70°F.

To determine the effectiveness of the bleaching agents and the extent to which the flours were bleached, flour pigment determinations were run on all the flour samples. A Wilkens-Anderson K. W. S. Z. photometer was used in connection with the method described by Binnington and Geddes (1939).

Experimental Results

The efficiency of the various bleaching treatments in reducing the yellow color in the flour is shown in Table III. No evidence of gray color was noticed from the heaviest bleach used. Nebred, a naturally highly pigmented variety, and Turkey, containing the second-highest amount of pigment, bleached readily with either of the bleaches employed.

TABLE III
AMOUNT OF FLOUR PIGMENT IN FOUR WINTER WHEAT FLOURS

Flour treatment	Tenmarq	Turkey	Nebred	Chuefkan
	<i>ppm</i>	<i>ppm</i>	<i>ppm</i>	<i>ppm</i>
None	1.74	2.49	2.95	1.73
Agene, 3 g per bbl	0.61	0.80	0.86	0.64
Agene, 9 g per bbl	0.33	0.49	0.41	0.43
Novadelox, 0.4 oz per bbl	0.90	1.21	1.56	0.75
Novadelox, 1.2 oz per bbl	0.26	0.46	0.54	0.36

Table IV presents loaf-volume data obtained from bread baked in these investigations. To reduce tabular material the bread scores for grain, texture, and crumb color have been omitted. It is interesting to note, however, that the optimum or best grain and crumb color scores normally were obtained at a bromate level 1 to 2 mg less than that required to obtain the largest or optimum loaf volumes, which are shown in bold-face type. Texture scores usually were improved by the use of milk in the formula. Crumb color was influenced by the amount of flour pigment remaining after bleaching.

Loaf-volume data are also shown graphically in Figures 1 (Agene-bleached flours) and 2 (Novadelox-bleached flours). These figures illustrate clearly the "buffering effect" of dry milk solids when relatively large amounts of bromate are used with or without bleaching. The figures also illustrate that the four varieties of wheat included in this experiment respond differently to the treatments imposed upon them.

It was observed that the flour pigment values tended to be reflected in the crumb color scores. It was desired to find whether the baking process itself might not also cause the removal of some color. Using unbleached Turkey flour, a number of bakes were made and the pigment content of the dried crumb determined. The baking treatments used and the results of the pigment determinations are presented in Table V. From these data it is evident that baking reduces the pigment content to some extent and that of the three factors varied, mixing time was the most effective.

Discussion

Examination of the data indicates clearly that the variety of wheat from which the flour was milled played an important part in the results obtained and in the conclusions which may be drawn from the investigations. The data show that the different varieties responded differently to bleaching treatment, to added bromate, and to added milk solids. In considering the results, the differences in amount of flour

TABLE IV
EFFECT OF BLEACHING AGENTS, POTASSIUM BROMATE, AND DRY MILK
SOLIDS UPON THE LOAF VOLUME OF FOUR WINTER WHEAT FLOURS

KBrO ₃ added mg	Loaf volume in cubic centimeters for each variety ¹							
	No milk solids				6% dry milk solids			
	Tenmarq	Turkey	Nebred	Chiefkan	Tenmarq	Turkey	Nebred	Chiefkan
NO FLOUR TREATMENT								
0	835	665	720	610	870	760	920	645
1	1060	920	910	840	—	—	—	—
2	1103	1053	960	960	—	—	—	—
3	1107	1075	910	1000	1188	—	1135	940
4	1007	1023	—	990	1255	1075	1178	960
5	—	—	—	—	1241	1140	1170	965
6	—	—	—	—	1237	1146	1153	980
7	—	—	—	—	—	1140	—	998
AGENE, 3 G PER BBL								
0	892	755	827	698	958	855	998	713
1	1123	985	905	873	—	—	—	—
2	1145	996	885	997	—	—	—	—
3	1020	958	820	1035	1220	—	1090	—
4	—	873	—	963	1223	1140	1075	970
5	—	—	—	—	—	1148	—	988
6	—	—	—	—	1217	—	1018	—
7	—	—	—	—	—	1155	—	1013
AGENE, 8 G PER BBL								
0	938	800	843	725	1028	898	1018	735
1	1103	980	920	905	—	—	—	—
2	1097	1008	863	1000	—	—	—	—
3	1012	993	790	980	1263	—	1060	—
4	—	880	—	963	1256	1160	1025	963
5	—	—	—	—	—	1135	—	990
6	—	—	—	—	1218	—	968	—
7	—	—	—	—	—	1105	—	1008
NOVADELOX, 0.4 OZ PER BBL								
0	900	713	763	658	978	805	935	683
1	1088	920	923	880	—	—	—	—
2	1168	1043	937	1000	—	—	—	—
3	1058	1020	885	1020	1190	—	1065	—
4	—	888	—	965	1263	1165	1110	978
5	—	—	—	—	—	1180	—	1033
6	—	—	—	—	1223	—	1016	—
7	—	—	—	—	—	1120	—	1015
NOVADELOX, 1.2 OZ PER BBL								
0	912	730	817	670	983	818	973	693
1	1120	913	947	880	—	—	—	—
2	1163	1065	955	1013	—	—	—	—
3	1047	1010	885	1013	1228	—	1125	—
4	—	910	—	977	1280	1143	1138	968
5	—	—	—	—	—	1192	—	1010
6	—	—	—	—	1253	—	1055	—
7	—	—	—	—	—	1165	—	985

¹ Bold-face type refers to optimum bromate level for maximum loaf volume.

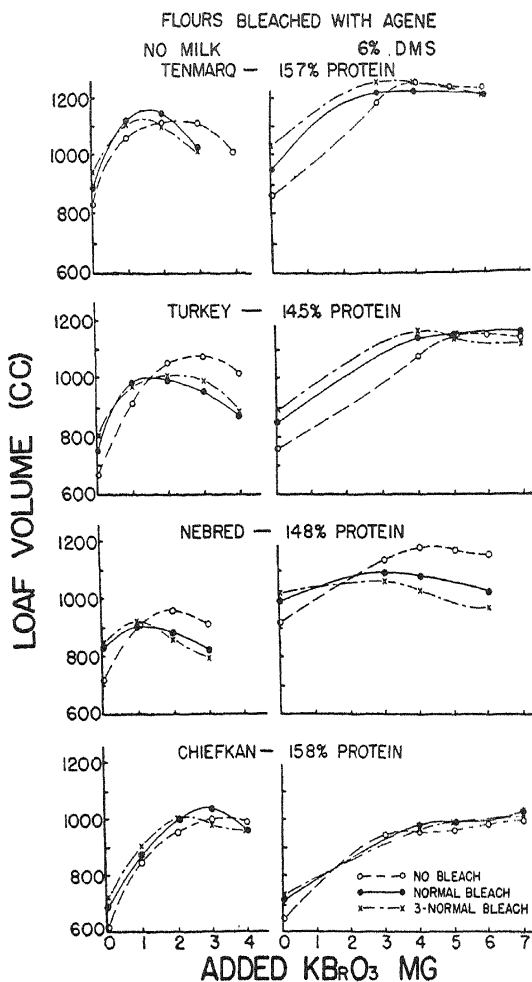


Fig. 1. The effect of potassium bromate upon the loaf volume of flours bleached with Agene.

protein must also be remembered. In this respect Tenmarq and Chiefkan were comparable, but they possessed a decided advantage over both Turkey and Nebred, which were approximately 1% lower in flour protein. Nebred had a slight advantage over Turkey but these two varieties may logically be compared.

Without potassium bromate, all flours responded favorably to

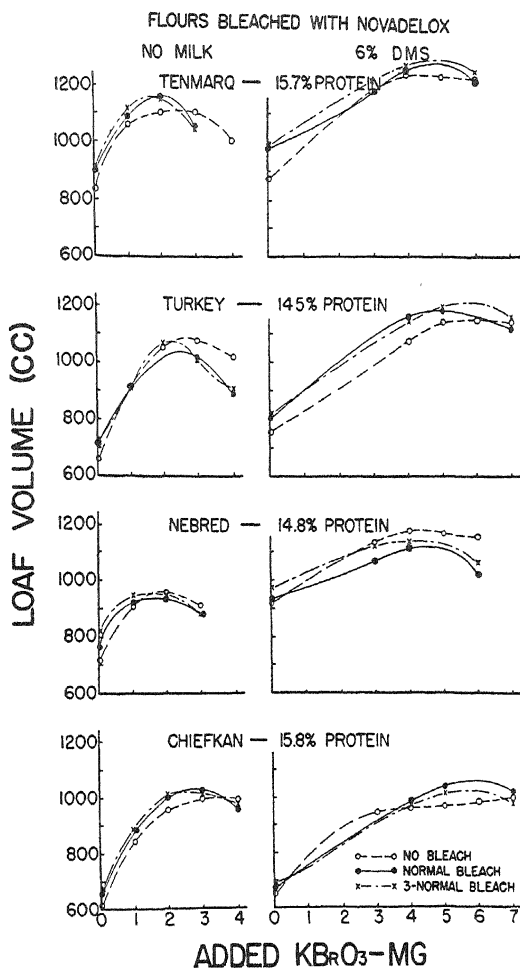


Fig. 2. The effect of potassium bromate upon the loaf volume of flours bleached with Novadelox.

bleaching, and the addition of 6% of dry milk solids to the formula produced still further improvement in the baked bread. Without bromate and without dry milk solids the Agene-bleached flours gave results superior to the Novadelox treatments. With added milk and no bromate, the Agene-treated Turkey was superior to the Novadelox-treated, but little apparent difference existed between the bleaching treatments with other varieties.

TABLE V
FLOUR PIGMENT CONTENT OF DRIED BREAD CRUMBS OBTAINED FROM
UNBLEACHED TURKEY FLOUR BAKED IN SEVERAL WAYS

Treatment	KBrO ₃ used mg	Flour pigment ppm
Flour before baking		1.19
Normal bake ¹	0	1.26
	1	1.40
	3	1.39
	5	1.48
	10	1.41
Fermentation time—1 hour	0	1.13
Fermentation time—5 hours	0	1.10
Mixing time—1 min	0	1.40
Mixing time—5 min	0	0.88

¹ Normal bake used 3-minute mixing time and 3 hour fermentation time.

Without bleaching treatment, Nebred responded very favorably to added dry milk solids, Chiefkan very little. It was evident that Nebred differed fundamentally from Turkey in respect to "milk requirement" for best baking performance. This sample of Nebred when baked without milk proved to be inferior to Turkey when the bromate requirement needed for optimum loaf volumes was used. Tenmarq responded favorably to dry milk solids without bromate but to a smaller extent than Nebred.

Generally speaking, increasing the amount of bleach produced an increase in loaf volume unless excessive amounts of bromate were used. The data indicate that Agene is somewhat more severe than Novadelox in its bleaching and maturing actions. This corroborates the earlier results obtained by Harris and Bayfield (1941). In most cases the bleaching agent reduced the amount of bromate necessary to attain maximum loaf volumes.

It was evident from the data that the addition of dry milk solids conferred considerable tolerance against the adverse effects of excessive quantities of bromate, whether the flours were bleached or not. An exception, however, must be made in the case of Nebred, which after bleaching gave definitely poorer results with or without milk at the optimum bromate level for the unbleached flour. It is unfortunate that the optimum bromate level was not attained in the Agene-treated Nebred series because of exhausting the flour supply. However, it was considered that a 2-mg bromate-milk bake would not have altered materially the general conclusions regarding this variety.

Compared with Tenmarq, the Chiefkan variety proved to be definitely inferior. Chiefkan proved to be remarkably lacking in ability to respond to the treatments given. All evidence to date shows that it possesses poor-quality gluten when compared to standard varieties such as Turkey or Tenmarq.

These investigations were undertaken to determine whether the use of dry milk solids would reduce the possibility of damage resulting from the overbromating of bleached hard winter wheat flour doughs. This is a question of considerable practical importance to the baking industry. The results obtained warrant an affirmative answer to the question.

Summary

A study was made with four winter wheat flours to ascertain the effectiveness of dry milk solids in preventing damage from overbromation in the baking of flour previously bleached with Agene or Novadelox. In addition, the combined as well as the individual effects of bleaching agents and potassium bromate were studied.

All samples, bleached or unbleached, were baked with or without 6% of dry milk solids. The flours were baked with amounts of potassium bromate ranging from 0 to 7 mg per 100 g of flour. Agene and Novadelox bleaching agents were used in normal and in 3-normal quantities.

Chiefkan showed little improvement from the addition of dry milk solids, and proved inferior to the other three varieties in baking quality. Nebred flour before and after bleaching proved to be very sensitive to added potassium bromate. Nebred benefited more than the other varieties from the addition of dry milk solids, although when bleached it gained little tolerance to bromation when milk was added. The superior baking quality of Tenmarq and of Turkey flours was again demonstrated.

Unbleached samples of Nebred and Turkey flours contained more pigment than samples of Tenmarq or Chiefkan. Normal treatment with Agene was more effective in the removal of color than the same treatment with Novadelox. The 3-normal treatment with either bleach reduced all varieties to approximately the same pigment content.

The four varieties studied varied in their response to added bromate, to dry milk solids, and to bleaching agent used.

The results obtained showed that the presence of 6% of dry milk solids in doughs reduced the possibility of damage from excessive amounts of bromate used in baking unbleached or bleached samples of flour. The inclusion of dry milk solids increased the loaf volume and improved the crust and crumb color, grain, and texture. Bread was appreciably improved by bleaching the flour but greater improvement resulted from the use of potassium bromate in the dough than from bleaching the flour.

Acknowledgment

The authors wish to express their appreciation to the Nebraska Agricultural Experiment Station and to the Kansas Wheat Improvement Association for providing most of the wheat samples used in these investigations. Thanks are also due to the American Dry Milk Institute, Chicago, Ill., for supporting their research assistant-ship under which a part of the work was done.

Literature Cited

- Balls, A. K., and Hale, W. S.
1936 Proteolytic enzymes of flour. *Cereal Chem.* **13**: 54-60.
- Barmore, M. A., Finney, K. F., and McCluggage, M. E.
1939 Quality characteristics of hard red winter wheat varieties grown in co-operative plot and nursery experiments in the hard red winter wheat region in 1938. U. S. Dept. of Agr., Bur. Plant Indus., Hard Winter Wheat Quality Laboratory, Manhattan, Kas. (Unnumb. Pub.), 26 pages, mimeographed.
- Binnington, D. S., and Geddes, W. F.
1939 A rapid method for the determination of wheat and flour pigments. *Cereal Chem.* **16**: 252-262.
- Eisenberg, Sylvan
1940 Evidence in support of a recent paper concerning the effect of milk on the bromate requirements of flours. *Cereal Chem.* **17**: 476-479.
- Finney, K. F., and Barmore, M. A.
1941 Study of baking methods for evaluating protein quality of hard winter wheats. Paper read at A. A. C. C. Convention, Omaha, Nebr.
- Geddes, W. F.
1930 Chemical and physico-chemical changes in wheat and wheat products induced by elevated temperatures. III. The influence of germ constituents on baking quality and their relation to improvement in flour induced by heat and chemical improvers. *Can. J. Research* **2**: 195-213.
- and Larmour, R. K.
1933 Some aspects of the bromate baking test. *Cereal Chem.* **10**: 30-72.
- Hanson, W. H.
1932 Effects of the amount and kind of bleach used on flour in relation to its aging. *Cereal Chem.* **9**: 358-377.
- Harris, M. C., and Bayfield, E. G.
1941 Bleaching agents vs. potassium bromate in baking with dry milk solids. *Cereal Chem.* **18**: 497-514.
- Jørgensen, Holger
1936 On the existence of powerful but latent proteolytic enzymes in wheat flour. *Cereal Chem.* **13**: 346-355.
- Larmour, R. K., Working, E. B., and Ofelt, C. W.
1939 Quality tests on hard red winter wheats. *Cereal Chem.* **16**: 733-752.
- Mohs, K.
1940 A study of flour treatment. *The Bakers' Digest* **14**: 163-164.
- Ofelt, C. W., and Larmour, R. K.
1940 The effect of milk on the bromate requirements of flours. *Cereal Chem.* **17**: 1-18.
- Rich, C. E.
1934 The chemical and physico-chemical changes induced in wheat flour by artificial maturing agents. *Cereal Chem.* **11**: 201-227.
- St. John, J. L., and Bailey, C. H.
1929 Effect of dry skim milk on the fermentation and hydrogen-ion concentration of doughs. *Cereal Chem.* **6**: 51-59.
- Sherman, H. C., Rouse, M. E., Allen, B., and Woods, E.
1921 Growth and reproduction upon simplified food supply. *J. Biol. Chem.* **46**: 503-519.
- Skovholt, O., and Bailey, C. H.
1932 Dry skim milk and new crop flours. *Northwestern Miller and American Baker* **9**: 18. Jan. 6.
- Sullivan, B., Howe, M., and Schmalz, F. D.
1936 On the presence of glutathione in wheat germ. *Cereal Chem.* **13**: 665-669.
- Working, E. B.
1928a The action of phosphatides in bread dough. *Cereal Chem.* **5**: 223-234.
1928b Some oxidizing effects of flour bleaching. *Cereal Chem.* **5**: 431-435.

BAKING TESTS AND THE EVALUATION OF NEW WHEATS¹

E. G. BAYFIELD and GLENN A. WEST

Kansas Agricultural Experiment Station, Manhattan, Kansas

(Received for publication February 2, 1942)

In the search for improved wheats a continuous stream of new varieties or strains flows to the cereal chemist for tests as to quality. These newer sorts are grown on small plots of land and frequently barely enough wheat is available for a small milling sample and a strictly limited number of bakings. The term "quality" has long been used in a broad sense, and without any standardized meaning. For the purposes of this discussion, however, "high or good quality" wheat will refer to a wheat which possesses properties making it well adapted for the market purposes of the class of wheat to which it belongs. The data reported at this time pertain to Kansas-grown wheats, and accordingly the quality designations will be used with particular reference to suitability for the market normally drawing a major part of its supplies from Kansas.

The bulk of Kansas-grown wheat consists of hard red winter varieties. From the standpoint of milling and baking qualities, the variety Turkey has, over the past several decades, proved widely acceptable as a strong bread wheat, and may be referred to as possessing good quality. Furthermore, other varieties possessing such properties or characteristics that they may be readily substituted for Turkey may also be considered as of good quality.

It must not be inferred that Turkey wheat, because of its excellent reputation for good quality, is the "perfect wheat" and may not be improved upon, because a wheat with Turkey qualities but without some of its deficiencies would undoubtedly find still wider acceptance by the trade. Turkey would be improved from a quality standpoint if it possessed heavier test weight per bushel with a consequent improvement in flour-yielding capacity, a somewhat lower amount of flour pigments, and a lower bromate requirement. Until new varieties with these advantages are produced, it is probable that wheat-quality improvement programs will continue. Notwithstanding room for improvements, Turkey does afford an acceptable standard of excellence in milling and baking quality. A new wheat possessing the properties of Turkey would readily find acceptance by millers using Southwestern wheats, and it is when new wheats differing widely from the accepted Turkey standards are distributed that complaints arise. To keep such

¹ Contribution No. 79, Department of Milling Industry.

complaints to a minimum and thereby maintain highest values to the grower, the use of the general principles outlined by Geddes (1941) is desirable for standardizing milling and baking qualities in new wheats distributed as a result of the efforts of the plant breeders.

Geddes (1941), in discussing the choice of new wheat varieties, states that: "The new variety should give satisfactory, and preferably optimum results in milling and baking when processed in the same manner as the 'standard variety' of the same wheat class. In this connection it is important to note that definite 'inferiority' in any one important quality characteristic is sufficient to render the variety unsuitable for release, even though it may be regarded as superior to the 'standard' variety in other particulars. Thus, higher flour-yielding ability cannot offset inferior baking properties, or good loaf volume, or undesirable dough characteristics."

Recently, in discussing baking results, Bayfield, Working, and Harris (1941) have commented on the need of care in choice of test procedures used in evaluating varieties so that varieties well adapted to consumer use may be selected. These workers found that two different baking methods tended to rank the varieties in different orders. More extensive data have been presented by Finney and Barmore (1941). This is not at all surprising, because any given baking procedure and formula will deviate more or less from the optimum for different samples. It is hardly conceivable that a baking technique will ever be found that is optimum for all samples because different varieties may be expected to respond in varying degrees to ingredients used in the baking formula and to physical operations involved. Fortunately it appears that some varieties possess the highly desirable capacity to give good baking results over a relatively wide range of baking conditions. Such varieties gain wide commercial acceptance because their flours are readily, and without specialized treatment, adapted to the different requirements of the bread-baking industry.

At this point it might be well briefly to emphasize the more important points to be considered in choosing a good baking test procedure. Today there appear to be two general philosophies governing the thinking of cereal chemists engaged in test baking. One is based on the development of methods whereby flours of different qualities and characteristics are made to give essentially the same baking results. This involves the adaptation of the baking test to the flour. A cereal chemist successful along these lines finds wide use of his skill as a "trouble shooter" in the baking industry. The second philosophy deals with methods whereby the flours are subjected to one or more uniform testing procedures in order to observe how well the flour fits into the requirements of a particular use. In this case a flour or wheat

variety must fit the testing procedure in order to be satisfactory. The authors believe that the latter general philosophy should govern in the variety testing program so as to eliminate undesirable wheats *before* they attain general distribution among the growers. The application of the former general philosophy to the variety testing program, it seems, will tend toward lack of uniformity in the quality of wheats grown in a given section of the country and cause much confusion for the milling and baking trades, which prefer, above all things, to produce a uniform product from day to day and throughout the year.

The baking test is probably the most important single test used in evaluating bread wheats. What are some of the requirements of a good baking test for *variety testing* work? The most important feature of any such baking test should be its tendency to rank or rate wheats in the order of their commercial acceptability. Unless the method employed will do this, it fails to achieve the major purpose of testing the samples. A second desired feature of almost equal importance would be that the method give as large a spread as possible between commercially desirable and undesirable varieties. This wide spread in results is needed so that differences between samples will not be due to errors or variations inherent in the baking test. A third feature is that the baking procedure should be practical and susceptible to "mass production" methods. In variety testing work large numbers of samples are customary, and intricate complicated testing procedures are not practical. Finally the formula should be as simple as it can be made because, as additional ingredients are included in the formula, variables are also added. There is always the possibility that such added ingredients may serve to cover up inherent weaknesses in a variety.

Numerous reports have been published dealing with the evaluation of baking qualities of new and old varieties of wheats. In spite of these reported results additional work is continually in progress as new methods and procedures necessitate the re-assessing of old and new wheats in the light of the improved techniques. The new wheats produced by the plant breeder, who often crosses rather diverse wheat types, introduce many new problems. These new wheats, as a result of their parentage, may frequently differ widely in characteristics such as mixing time, absorption, milling properties, flour granulation, bromate requirement, milk requirement, and so forth. These wide differences make the problem of finding a "perfect" or universal baking method adapted to all wheats increasingly unlikely. It is questionable if any formula and technique can give optimum bread results over the range of baking qualities encountered within one class of wheats, and it seems rather improbable that a given method can even be considered

as giving "optimum" baking results over the entire protein range within one variety.

The method which aims at producing optimum bread over a wide range in flour properties requires a high degree of skill based upon years of experience. Such a "skilled" baking method would entail varying formulas, fermentation times and temperatures, dough handling and other features of the baking process to suit each sample, and would not meet the requirements of the cereal chemist who for many years has striven diligently for more science and exactness in baking. While the application of the optimum principle to baking would give information as to requirements for making good bread from any flour, it is very doubtful if such information is needed for new varieties still in the hands of the wheat breeder, because the industry cannot readily change commercial bread making to suit a wide range in flour properties. The baker prefers flour that fits into the baking methods current in his shop. The miller therefore must buy wheat which will make the desired type of flour. To fit market needs and provide a good market for the wheat grower, it is therefore imperative that the plant breeder produce wheats which will find ready use by miller and baker. In this scheme of things the cereal chemist needs baking procedures which will accurately eliminate the unwanted varieties.

It has already been indicated that flour milled from Turkey wheat finds a ready market, and that in spite of its faults this variety may be used as a standard or base for comparison against which new varieties can be rated. Varieties which respond similarly to Turkey may be expected to fit the requirements of markets which now use Turkey flours. Varieties which do not respond similarly to Turkey may be suited to bread production but they will not be equally suited to the market needs. Good bread can be made from some soft winter wheat flours, yet this does not make these flours equally useful in a market accustomed to hard red winter wheat flour.

Recently, Parker and Bayfield (1941) reported upon a collaborative preference rating of quality for Turkey, Tenmarq, Blackhull, and Chiefkan wheats or flours milled from these wheats. Twenty-three laboratories ranked either Turkey or Tenmarq first, two preferred Blackhull as first choice, and one laboratory indicated Turkey or Tenmarq equal to Blackhull in first place, depending upon anticipated use of the flours. Chiefkan was a very poor fourth in order of preferences. The four wheats were grown under comparable conditions in acre plots. As the baking methods employed by these collaborators differed widely, the agreement in final opinions was surprisingly good. This study indicates that there should be no question as to the suitability of Turkey as a standard of comparison. Tenmarq, with a

lower pigment content and lower bromate requirement, may be considered superior to Turkey in these respects and its equal otherwise as a bread wheat.

The 1938 crop studies of Larmour, Working, and Ofelt (1939, 1940) and those for 1939 of Bayfield, Working, and Harris (1941) show that some varieties of hard red winter wheat are relatively poorer for bread production than some soft winter wheats. These studies in the present instance have been continued on the 1940 crop by the authors.

Materials and Methods

Small lots of grain of pure varieties were obtained ² from widely scattered tests over the state of Kansas. These samples varied considerably in protein content as they originated from some 43 different locations. Hard red winter varieties included were Turkey, Tenmarq, Kanred, Cheyenne, Nebred, Early Blackhull, Blackhull, Chiefkan, Iobred, Iobred Selection, Pawnee (Kawvale x Tenmarq), and Commanche (Oro x Tenmarq); whereas Kawvale (semihard), Michigan Wonder, and Clarkan represented the soft red winter varieties.

After all unsound samples as well as those weighing less than 53 pounds per bushel were discarded, the protein content of each was determined. The samples were then composited according to variety so as to give, at different protein levels, composites which were sufficiently large for milling and baking tests. In making the required wheat mixes the following wheat protein levels were desired:

Below 10%	14.6-16.0%
10.1-11.5%	16.1-17.5%
11.6-13.0%	17.6% and above
13.1-14.5%	

Each variety was not grown at all locations and it was not possible to have all protein levels represented in each variety. By blending the samples on a protein basis without regard to location it was hoped to overcome the effect of environmental growing conditions. The composited samples were thoroughly mixed and then milled on a Buhler mill. The resulting straight-grade unbleached flours were analyzed, stored at room temperature in tightly covered cans for six weeks, and then removed to a refrigerated room until the baking tests were completed.

Analytical and baking procedures used in this study were similar to those given earlier by West and Bayfield (1942). In the present study four different formulas were used, and the given average loaf-volume scores resulted from two or more single loaves, each baked on

² The authors acknowledge their appreciation to A. L. Clapp of the Department of Agronomy for supplying these samples.

different days. The individual bakes were replicated until loaves checking within 20 cc were obtained. All samples were baked with Formula 1 (referred to as "rich" formula) and Formula 2 (malt-phosphate-bromate or MPB formula) while four varieties—Turkey, Tenmarq, Blackhull, and Chiefkan—were also baked with Formula 3 (modified MPB) and Formula 4 (modified rich formula). Optimum mixing times were used throughout. The composition of these formulas is given in Table I.

TABLE I
FORMULA INGREDIENTS

Ingredient (per 100 g flour)	Formula			
	1	2	3	4
	g	g	g	g
Flour ¹	100	100	100	100
Sugar	6	5	5	6
Salt	1.5	1	1	1
Yeast	2	3	2	3
Dry milk solids (DMS) ²	6	—	—	6
Shortening	3	—	—	3
Malt syrup—120° L	0.25	—	—	0.25
Malt syrup—200° L	—	0.30	0.30	—
NH ₄ H ₂ PO ₄	—	0.10	0.10	—
KBrO ₃	0.004	0.001	0.002	0.004
Water	As needed	As needed	As needed	As needed

¹ Weighed out on 15% moisture basis.

² Containing not over 1½% fat and not over 5% moisture.

With the exceptions that 6% instead of 4% dry milk solids and 4 mg instead of optimum bromate were used, Formula 1 (rich formula) was identical with that used extensively by K. F. Finney in the Hard Winter Wheat Quality Laboratory of the U. S. Department of Agriculture at the Kansas Agricultural Experiment Station. Formula 2 (MPB), the malt-phosphate-bromate formula, is used extensively by spring wheat investigators. Formula 4 (modified rich) was the formula employed by Bayfield, Working, and Harris (1941) on Kansas-grown 1939 crop samples. Formula 3 (modified MPB) was included to study the effect of using less yeast and more bromate in the MPB formula, which, it was thought, might penalize varieties with a bromate requirement higher than 1 mg.

Results and Discussion

Table II presents both analytical and baking data for the different variety samples. These data give, among other things, information regarding the possible usefulness of the MPB formula for hard red winter wheats. The wheats provide a wide range of protein contents,

as well as widely differing baking qualities. It was desired to find whether this formula would give essentially linear relationships between loaf volume and protein content, particularly with varieties such as Blackhull or Turkey, which are commonly considered as possessing high bromate requirements. Recently Larmour (1941) has reported favorable results from the use of this formula when used on hard red winter wheats. Bayfield (1941) by using one-half the stipulated quantities of malt, phosphate, and bromate in this formula obtained satisfactory results in testing flours of soft winter wheat varieties. Bayfield and Shiple (1937) concluded that the use of the full amounts of malt, phosphate, and bromate was too severe for normal strength flours of soft winter wheat.

Results in Table II indicate that the same flour when baked by different formulas gave different loaf volumes. Some samples gave relatively larger or smaller volumes with certain formulas than with others. This means that the relative rankings of the varieties tend to change with baking formula as a result of differences in response to formula ingredients. As all varieties included in this study are not equally desirable from a utility or industrial standpoint, it obviously is essential that the proper formula be chosen; otherwise there is the danger that undesirable varieties will be awarded a higher ranking than they merit.

The varieties Turkey, Blackhull, Tenmarq, and Chiefkan were baked by all four formulas, preliminary to baking the other varieties, in order to observe whether Formula 3 might not prove superior to Formula 2 and whether Formula 4 would give similar or better results than Formula 1. Figure 1 shows the regression lines of loaf volume on protein content of flour when the above-mentioned varieties were baked by all four formulas. All four formulas indicated Chiefkan to be definitely of poor baking quality, thus confirming results obtained by Larmour, Working, and Ofelt (1939) and Bayfield, Working, and Harris (1941) on the 1938 and 1939 crops respectively. With all four formulas, Turkey gave satisfactory results, an indication that this variety possesses the very desirable characteristic of being reasonably tolerant to formula changes. However, the relative behavior of Turkey at different protein levels is changed by substituting Formula 4 in place of Formula 1.

The relative positions of Tenmarq and Blackhull with the different formulas are interesting and of considerable importance. It has already been indicated that a very large proportion of the trade prefers Tenmarq to Blackhull. The formulas containing milk and shortening indicate Blackhull as superior to Tenmarq. It was thought that possibly the use of 4 mg of KBrO_3 was depressing the baking performance of the Tenmarq. However, when the Tenmarq series was

TABLE II
ANALYTICAL AND BAKING DATA

Serial No.	Test weight ¹	Flour yield ²	Wheat protein ³	Flour ash ³	Flour protein ³	Absorption ^{3,4}	Loaf volume and mixing time ⁵ with formula			
							1	2	3	4
	lbs	%	%	%	%	%	min	cc	min	cc
TURKEY CI 1558										
829	59.0	70.1	9.0	0.38	8.2	55.3	4 1/4	673	4	590
830	58.8	69.0	10.5	0.39	9.3	56.1	4	773	3 3/4	663
831	59.0	70.3	11.3	0.40	10.2	56.8	3 3/4	835	3 1/2	695
832	58.3	71.7	13.1	0.41	11.8	58.5	3 1/2	944	3 1/4	825
833	57.0	71.4	14.3	0.44	13.3	60.0	3 1/4	988	3 1/4	843
834	54.2	70.7	16.8	0.45	15.5	63.7	3 3/4	1173	3 3/4	1078
TENMARQ CI 6936										
823	57.3	72.2	8.8	0.40	7.9	54.6	4 3/4	613	4 1/2	558
824	59.3	73.3	10.4	0.39	9.3	57.2	4 1/4	677	4 1/4	615
825	58.5	73.3	11.6	0.37	10.8	58.2	3 3/4	780	4	707
826	58.7	72.9	12.8	0.38	11.8	60.1	3 3/4	838	3 1/2	773
827	57.6	71.9	14.1	0.36	13.3	61.0	3 3/4	893	3 1/4	833
828	55.0	71.6	15.9	0.43	15.3	64.0	3 3/4	1118	3 1/2	1045
BLACKHULL CI 6251										
764	60.6	70.0	9.4	0.35	8.4	52.5	3 1/4	695	3 1/4	590
765	62.2	70.4	10.4	0.35	9.1	53.3	2 1/2	753	3	613
766	62.0	70.5	11.8	0.35	10.5	54.1	2 3/4	825	2 1/4	693
767	60.3	70.0	13.1	0.36	11.8	55.1	2 3/8	913	2 1/4	763
768	60.2	72.3	14.1	0.38	13.1	57.2	2 1/2	940	2 1/4	795
769	57.3	70.6	15.9	0.41	15.1	59.0	2 3/4	1110	2 1/4	980
770	58.3	70.2	18.1	0.42	17.4	62.7	2 5/8	1193	2 1/4	1085
CHIEFKAN CI 11754										
780	62.2	74.0	9.7	0.42	8.7	54.4	2 1/4	640	2	558
781	62.0	74.7	10.4	0.41	9.3	55.5	2 1/2	648	2	595
782	61.5	74.9	11.9	0.37	10.7	56.2	2 3/8	720	1 3/4	662
783	61.6	74.4	12.9	0.37	11.6	58.5	2 3/8	735	1 3/4	683
784	60.2	72.2	14.4	0.39	13.1	59.3	2	785	1 3/4	758
785	60.1	72.7	14.9	0.43	14.6	61.1	2	840	1 3/4	763
KANRED CI 5146										
797	59.4	74.8	9.6	0.36	8.6	55.0	3 7/8	698	3 3/4	585
798	58.0	71.4	10.1	0.36	8.9	55.8	3 3/4	708	3 3/4	645
799	59.3	75.0	11.6	0.36	10.4	56.3	3 1/2	785	3 1/4	685
800	57.6	73.8	13.2	0.38	12.0	57.1	3 1/8	903	2 3/4	810
801	57.8	73.5	14.5	0.41	13.3	58.8	2 1/2	937	2 1/4	823
802	56.2	72.4	16.3	0.43	15.5	60.6	2 1/4	998	2 1/4	907
803	55.0	74.3	18.0	0.46	17.4	64.8	2 3/4	1110	2 3/4	985
EARLY BLACKHULL CI 8856										
771	62.2	70.8	11.6	0.35	10.9	52.7	2 5/8	798	2 1/4	680
772	61.8	71.4	13.2	0.36	12.4	54.8	2 1/8	845	1 3/4	765
773	61.2	72.9	13.5	0.35	12.7	55.8	2	840	1 1/2	720
774	60.9	72.7	15.4	0.37	14.7	58.6	2 1/4	935	1 3/4	820
CHEYENNE CI 8885										
775	60.5	73.9	10.3	0.37	9.2	55.2	6	685	5 1/4	608
776	59.2	70.8	11.6	0.37	10.4	57.2	5 3/4	743	4 3/4	682
777	57.2	72.7	12.3	0.40	11.2	58.1	5 1/4	820	3 3/4	705
778	59.9	75.6	14.4	0.44	13.2	59.5	4 1/2	883	3 3/4	775
779	57.9	74.2	15.8	0.44	14.8	64.4	5 1/2	953	4 1/2	828
779A	56.6	74.8	16.8	0.50	15.6	65.2	5 1/2	1010	4 1/2	975

TABLE II—Continued

Serial No.	Test weight ¹	Flour yield ²	Wheat protein ²	Flour ash ³	Flour protein ³	Absorption ^{3,4}	Loat volume and mixing time ⁵ with formula					
							1		2		3	4
	lbs	%	%	%	%	%	min	cc	min	cc	cc	cc
IOBRED CI 6934												
791	62.2	74.0	10.8	0.34	9.6	54.4	4	703	3½	618	—	—
792	61.9	72.5	12.6	0.32	11.3	55.4	3½	830	2¾	718	—	—
793	61.2	75.5	14.0	0.34	13.0	56.5	3	953	2½	845	—	—
IOBRED SELECTION CI 11997												
794	62.2	75.7	10.6	0.34	9.7	53.5	4½	720	3½	605	—	—
795	59.7	71.4	12.8	0.33	11.1	54.4	3¼	868	2¾	740	—	—
796	61.1	72.8	14.4	0.32	13.4	57.2	3	990	2½	868	—	—
PAWNEE CI 11669												
809	59.2	74.5	10.0	0.36	8.9	51.9	3	705	3	602	—	—
810	60.9	73.8	11.7	0.33	10.6	54.9	3	773	3	673	—	—
811	60.9	73.9	13.7	0.31	12.3	55.4	2½	847	2¾	728	—	—
813	56.8	72.3	17.2	0.40	15.9	59.7	2½	1128	2½	1118	—	—
COMANCHE CI 11673												
820	59.5	73.0	10.8	0.41	10.1	56.3	3½	755	4	660	—	—
821	58.9	76.5	12.7	0.43	11.8	59.2	4	855	4	795	—	—
822	56.9	72.0	15.7	0.45	14.7	63.1	3¼	1007	2¾	955	—	—
NEBRED CI 10094												
818	60.3	71.4	14.4	0.38	13.0	59.8	3½	930	2¾	828	—	—
819	57.3	71.0	16.1	0.43	15.2	62.4	3¾	1062	3¼	985	—	—
KAUVALE CI 8180 (SEMI-HARD, SOFT RED WINTER)												
804	58.1	73.6	9.5	0.40	8.3	52.6	3½	650	3½	560	—	—
805	58.3	73.2	10.4	0.39	8.9	52.5	3¼	700	3¼	597	—	—
806	60.0	72.0	11.7	0.38	10.4	54.7	3¼	808	3	692	—	—
807	57.7	73.7	13.0	0.39	11.7	57.3	2¾	857	2¾	740	—	—
808	57.8	71.0	14.9	0.38	13.3	58.1	2¾	1028	2¾	928	—	—
MICHIGAN WONDER CI 5620 (SOFT RED WINTER)												
814	56.3	65.2	9.4	0.37	8.3	48.2	3½	647	2¾	532	—	—
815	57.7	65.0	10.2	0.36	8.7	49.1	3¼	693	2½	583	—	—
816	59.2	68.4	10.6	0.36	9.0	50.3	3½	707	2½	588	—	—
817	56.9	64.4	12.9	0.34	10.8	52.3	3	780	2¼	715	—	—
CLARKAN CI 8858 (SOFT RED WINTER)												
786	61.0	65.2	9.7	0.32	8.3	52.3	2½	683	1¾	538	—	—
787	60.5	67.0	10.2	0.31	8.7	53.2	2½	725	1¾	570	—	—
788	60.9	66.8	11.4	0.31	9.9	54.6	2¼	768	1¾	638	—	—
789	60.9	66.8	12.8	0.32	11.3	55.2	2	798	1½	695	—	—
790	60.8	65.0	14.6	0.35	12.8	55.3	1½	812	1½	700	—	—

¹ Cleaned weights.² On "as received" basis, not corrected to uniform moisture basis.³ Moisture basis 15%.⁴ Absorption used for Formulas 2 and 3, Formulas 1 and 4 values 4% higher.⁵ Mixing times: Formulas 1 and 4 the same, 2 and 3 the same

rebaked using either 2 or 3 mg of bromate in the rich formula, it was found that this formula still consistently placed Blackhull ahead of Tenmarq. The modified MPB formula indicates that Blackhull is equal to Turkey and much superior to Tenmarq. The MPB formula, of the four formulas employed, placed Tenmarq the closest to the

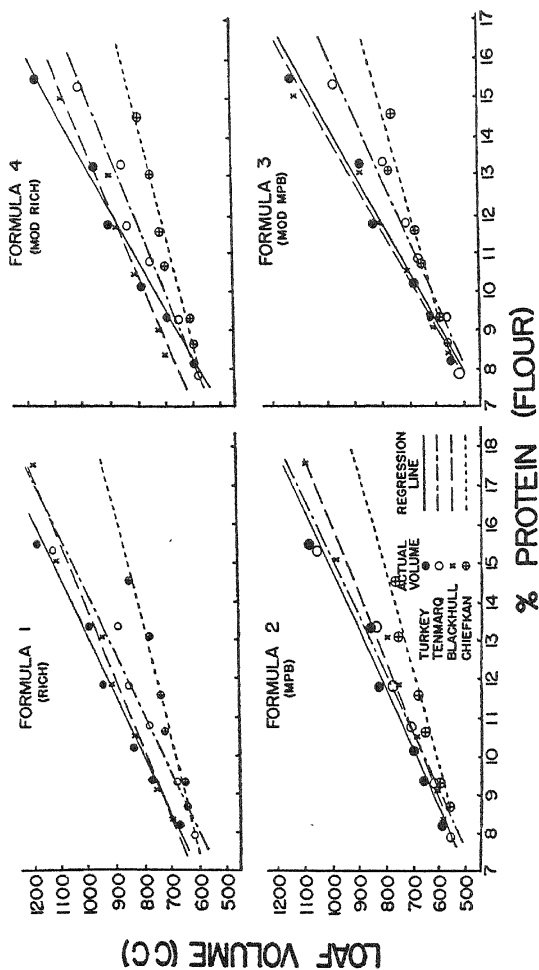


Fig. 1. The effect of four formulas upon loaf volume-protein regression lines of four widely different quality varieties

equally desirable variety Turkey. Unfortunately this formula has the disadvantage of a relatively small spread between varieties. While spread between varieties is highly desirable, correctness of ranking is of paramount importance.

The baking data from these four varieties were sufficiently promising to warrant a comparison of the MPB formula with the rich formula on the remainder of the varieties. Figure 2 shows the regression lines

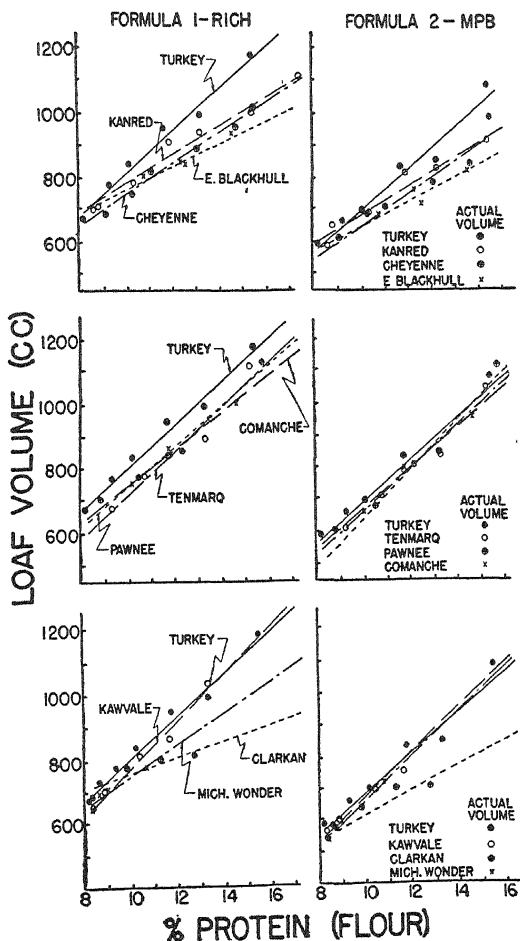


Fig. 2. A comparison of loaf volume-protein regression lines obtained from Turkey and several other varieties.

calculated from loaf volume and protein data for the remainder of the 14 varieties. Turkey has also been included for purposes of comparison. As only two protein levels were available for Nebred, no regression line is shown for this variety. The actual baking results with this variety show that with the rich formula Nebred possessed no particular advantage over Tenmarq of approximately equivalent protein content, while with the MPB formula the former was, if anything, inferior to the latter variety. At these protein levels Turkey gave larger volumes than Tenmarq with either formula. The behavior of these two Nebred samples seems to confirm the earlier results of West and Bayfield (1942), who concluded that Nebred needed milk in the formula to bring out its potential baking strength.

Among the minor acreage varieties grown in the hard red winter section of Kansas, Early Blackhull, Cheyenne, and Chiefkan are shown in Table II as possessing poor baking qualities. Kanred, which now occupies only a relatively small acreage, possessed only a fair baking performance in the 1940 crop tests. Additional experience is required with the newer hard winter varieties, Iobred, Iobred Selection, Comanche, and Pawnee, but in this particular year they gave promising baking results.

Among the varieties grading soft red winter, considerable differences in baking performance may be observed. Kawvale, which *looks* like soft red winter wheat, is seen to possess excellent baking quality, ranking with Turkey. As such baking performance is not desired in a soft red winter variety, this indicates *poor* quality in this variety as a *soft* wheat. The remaining two soft winter varieties, Michigan Wonder and Clarkan, are apparently distinctly different in the baking characteristics. This difference was noted by Bayfield, Working, and Harris (1941) in reporting upon the 1939 samples.

A number of interesting points may be observed from the data in Table II. In a general way it may be observed that loaf volume, absorption, flour ash, and flour protein tend also to increase with increasing protein content of the wheat, whereas test weight and mixing time tend to decrease. Flour yield shows a slight tendency to decrease with increasing protein content. While micro-mixer curves are not presented in this paper they were made on all the samples and these curves indicated shorter times to the peak of development and greater heights to the peak as the amount of protein increased. The curves bore a strong resemblance to those published earlier for the 1939 crop samples.

Comparison of the individual varieties shows definite differences in some respects. It may be noted that Blackhull, Early Blackhull, and Clarkan apparently give relatively less flour for a given test weight than varieties such as Turkey, Tenmarq, or Cheyenne. The former

three varieties yield low-ash flours. The high-test-weight variety Chiefkan is a relatively better flour-yielding sort than Blackhull. Iobred apparently is a low-flour-ash variety, whether the protein content is high or low. It is evident that milling qualities must be considered separately from baking characteristics. A variety may possess merit from a milling standpoint and be poor from a baking standpoint, or the reverse. A desirable variety needs to possess merit for both the miller and the baker.

The Regression Coefficient

The regression coefficients for loaf volume on flour protein were calculated for the different varieties when baked by the different formulas. The resulting information was used in preparing the regression lines shown in Figures 1 and 2. Except for the variety Clarkan, examination of the loaf volume and protein data indicate that these two factors are in an essentially linear relation.

McCalla (1940), working with spring wheats, has indicated that the loaf volume-protein content regression coefficient is a varietal characteristic which is inherited. He used a baking procedure adapted to wheats grown in Western Canada. Using Formula 4 with both a fixed and an optimum mixing time Bayfield, Working, and Harris (1941) found that the regression coefficient varied with varieties of hard winter wheat. Furthermore the coefficient was changed somewhat depending upon the mixing procedure used.

In the present study, using optimum mixing times, the formula has been varied, and, as shown in Figures 1 and 2, this change in formula frequently changed the slope of the regression line. It seems likely that, had further changes been made in formula, additional changes in the regression lines would also have resulted. As long as the regression lines are essentially parallel, no change in relative ranking of varieties will result. Unfortunately these lines frequently are not parallel for all varieties, and this is due it would seem to differences between varieties in response to baking ingredients or baking manipulations.

Barmore, Finney, and McCluggage (1941), working with hard red winter varieties, employed a formula similar to Formula 1 but with 2% less dry milk solids and sufficient bromate to give maximum loaf volume. They have employed the loaf volume-protein regression in computing a protein-quality correction factor. This procedure in the hands of these workers showed the variety Nebred to be exceptionally good as compared to the other varieties included in their study. Their method appeared to rank other commonly grown hard red winter wheats approximately as accepted commercially. The use of the optimum bromate level in the evaluation of new varieties introduces

a serious handicap. This is due to the frequent scarcity of flour and to the necessity for making a series of tests to determine the optimum bromate level for each sample.

Figures 1 and 2 show that the slope and relative height of the regression line may vary with the formula used. If a formula adapted to a good standard variety is used upon a series of varieties, it would seem that those varieties which gave regression coefficients and regression lines similar to that of the standard variety would also fit the market requirements met by the standard variety. On the other hand, varieties which gave regression lines much different from the standard when baked with the standard formula might be expected to cause trouble for the miller and baker. Similar trouble could be expected from varieties which gave regression lines similar to the standard variety only after given some special formula or other treatment. Such special treatments might include unusually short or long mixing times, special flour bleaching treatments, extra long or short fermentation times, or unusual requirements (in terms of the accepted standard) in any of the baking ingredients.

Summary

From a study of several hard and soft winter wheat varieties grown in Kansas in 1940 it is shown that choice of baking formula plays an important part in deciding which variety is most likely to suit the market requirements.

The possible use of the regression coefficient of loaf volume upon protein content as a protein-quality factor is discussed and it is shown that the type of regression line is dependent upon baking formula as well as upon variety.

It is concluded that in the *variety testing* program it is preferable to test new varieties by a method found to be adapted to a variety which is widely acceptable industrially. Those new varieties which do not respond in a manner similar to that of the standard variety are likely to prove unacceptable to the milling and baking industries even if they do possess otherwise potentially desirable characteristics.

Literature Cited

- Barmore, M. A., Finney, K. F., and McCluggage, M. E.
 1941 Quality characteristics of hard red winter wheat varieties grown in co-operative plot and nursery experiments in the hard red winter wheat region in 1939. U. S. Dept. Agr., Bur. Plant Indus., Div. Cereal Crops and Diseases (unnumb. pub.) (mimeographed).
 Bayfield, E. G.
 1941 Report on baking test for soft wheat flours. J. Assoc. Offic. Agr. Chem. 24: 590-611.
 — and Shiple, V.
 1937 Soft winter wheat studies. V. Evaluating the quality and the strength of some varieties. Cereal Chem. 14: 551-577.

- Working, E. B., and Harris, M. C.
1941 The effect of protein content on the baking behavior of some winter wheat varieties. *Cereal Chem.* 18: 640-654.
- Finney, K. F., and Barmore, M. A.
1941 A study of baking methods for evaluating the protein quality of hard winter wheats. Paper presented at A. A. C. C. convention, Omaha, May, 1941.
- Geddes, W. F.
1941 Objectives in breeding for improved quality in hard wheat. *J. Am. Soc. Agron.* 33: 490-503.
- Larmour, R. K.
1941 A comparison of hard red spring and hard red winter wheats. *Cereal Chem.* 18: 778-789.
- Working, E. B., and Ofelt, C. W.
1939 Quality tests on hard red winter wheats. *Cereal Chem.* 16: 733-752.
1940 Quality tests on soft red winter wheats of Kansas. *Cereal Chem.* 17: 18-29.
- McCalla, A. G.
1940 Varietal differences in the relation between protein content of wheat and loaf volume of bread. *Can. J. Research* 18C: 111-121.
- Parker, J. H., and Bayfield, E. G.
1941 Milling and baking tests on Tenmarq, Turkey, Blackhull and Chiefkan wheats grown uniformly on acre plots. Paper presented before the Tri-section Meeting, A. A. C. C., Manhattan, Kansas, April, 1941.
- West, G. A., and Bayfield, E. G.
1942 Effectiveness of dry milk solids in preventing overbromation of some bleached hard winter wheat flours. *Cereal Chem.* (previous paper, this issue).

THE EFFECT OF HEAT TREATMENT OF MILK IN RELATION TO BAKING QUALITY AS SHOWN BY POLAROGRAPH AND FARINOGRAPH STUDIES¹

OLOF E. STAMBERG² and C. H. BAILEY

Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota

(Received for publication August 27, 1941)

It is well known that fresh milk and milk products such as condensed or dried milk which have not been subjected to a sufficient heat treatment have poorer bread-baking qualities than properly heated milk. Numerous theories have been advanced as explanations for this effect of the heat treatment, such as the destruction of proteolytic enzymes, changes in the colloidal properties of the salts, denaturation and coagulation of the proteins, and changes in the oxidation-reduction systems.

Investigations in this laboratory indicate that the presence of sulfhydryl groups in unheated milk appears to be the major cause for its dough-softening or degrading action. Very small amounts of cysteine or glutathione will produce a rapid degradation of a dough. This action has been explained as due to the activation of proteolytic

¹ Paper No. 1917, Scientific Journal Series, Minnesota Agricultural Experiment Station.

² Formerly American Dry Milk Institute Research Associate; present address, Chemistry Department, Stanford University, Palo Alto, California.

enzymes present in flour and also as due to a direct action on some flour constituent. The latter appears quite probable since the action of proteolytic enzymes in quantities sufficient to produce a poor-quality loaf of bread is slow, while equivalent quantities of certain sulphydryl compounds produce a rapid dough-softening action readily observed during mixing. Such observations were also made by Balls and Hale (1936), Ford and Maiden (1938), and Freilich and Frey (1939).

Baerstein (1936) reported the sulfur content of milk proteins and the distribution as methionine and cystine as follows:

Protein	Percent sulfur	Percent of total sulfur as*	
		Cystine	Methionine
Casein	0.80	9.8	83.4
Lactalbumin	1.45	62.1	34.4

This indicates that milk proteins are potentially high in organic groups containing sulphydryl groups.

Brdicka (1933) used the polarograph for the determination of cystine or cysteine in a buffered solution containing bivalent cobalt and found a current-voltage maximum at 1.5 volts. A current voltage curve is thus obtained which is characteristic of the sulphydryl group or the disulfide linkage in cystine, the latter being first reduced to the sulphydryl group at the mercury cathode. It is considered that the cobalt salt forms a complex with the sulphydryl compound and that the characteristic current-voltage curve depends upon the catalytic reduction of the hydrogen of the sulphydryl group.

Smith and Rodden (1936) found that methionine showed no reduction wave typical of cysteine or cystine. Mohr and Wellm (1936) prepared different protein fractions from milk and made polarographic analyses in the presence of hexamine-cobaltic chloride, which was used in the study of the protein effect. Somewhat different curves were obtained for various fractions, but they did not attempt to interpret these differences. They did not use cobaltous chloride, described by Brdicka as specific for the detection of sulphydryl compounds.

In this study, polarographic analyses were made of heated and unheated fresh separated milk samples together with tests in the farinograph.

Experimental

A series of tests was made on milk using the Heyrovsky micro-polarograph. The instrument is an automatic device designed to provide a photographic record of the current voltage curves in dropping mercury cathode analysis. A regularly increasing voltage is applied to the electrolytic cell, and the change in current flowing through the cell, as detected by a highly sensitive galvanometer, is automatically recorded on photographic paper as a function of the applied voltage.

The milk used in all of the tests was unpasteurized separated milk which was not over 30 hours old, and when reference is made to heated milk the heat treatment involved was holding the milk at 97°–100°C for five minutes. Several tests were made on milk from different lots.

A series of tests made with different final milk concentrations indicated that 0.4% in the final reaction mixture was an effective concentration to use. The curves obtained with 0.8% concentration were also easily reproduced, but the lower concentration was found to be better as a result of the high sensitivity of the instrument.

For polarograms the following solutions were quickly mixed in a test tube:

1.0 ml milk diluted 1–25 with water, or 1 ml 0.0001 *M* cystine for the standard,
0.5 ml 0.05 *M* cobaltous chloride,
2.0 ml buffer, 0.5 *N* NH_4Cl and 0.5 *N* NH_3 ,
6.5 ml distilled water.

This makes the final dilution of the milk 1–250 or 0.4%. The mixture was immediately introduced into the electrolytic cell and the measurements started. The solution must be used at once since standing causes alkaline decomposition of the milk proteins.

The results obtained with four samples of milk from different supplies are shown by the curves reproduced in Figure 1. The top curve was obtained by using 1 ml of 10^{-4} *M* cystine solution. The four curves to the left are from the heated milk samples and to the right are the corresponding curves of the raw unheated milk samples. It is clearly noticeable that with the unheated milk a maximum (at point A) occurs which is not present after heat treatment. Since, according to Brdicka (1933), this maximum is specific for organic sulfhydryl compounds, such as cysteine, in the presence of cobaltous chloride, it appears that heat treatment eliminates such groups present or exposed in the raw milk.

Three typical curves for cystine, raw milk, and heated milk are plotted in Figure 2, showing the galvanometer deflection at the calculated applied voltage. The maximum for the cystine at 1.5 volts is in agreement with Brdicka, while the maximum for the raw-milk curve is at approximately 1.43 volts. This might be due to the sulfhydryl groups in the milk being attached to the protein units and hence showing a maximum at a slightly different voltage than for free cysteine. In the case of the raw-milk curves a slight maximum was also observed at about 1.2 volts.

Figure 3 shows how the curves can be used to estimate quantitatively the concentration of the sulfhydryl groups as compared with the cystine curve from a known concentration. The minimum in the curve just past the maximum is measured. The first maximum noticed in all curves is due to the reduction of the cobaltous chloride at

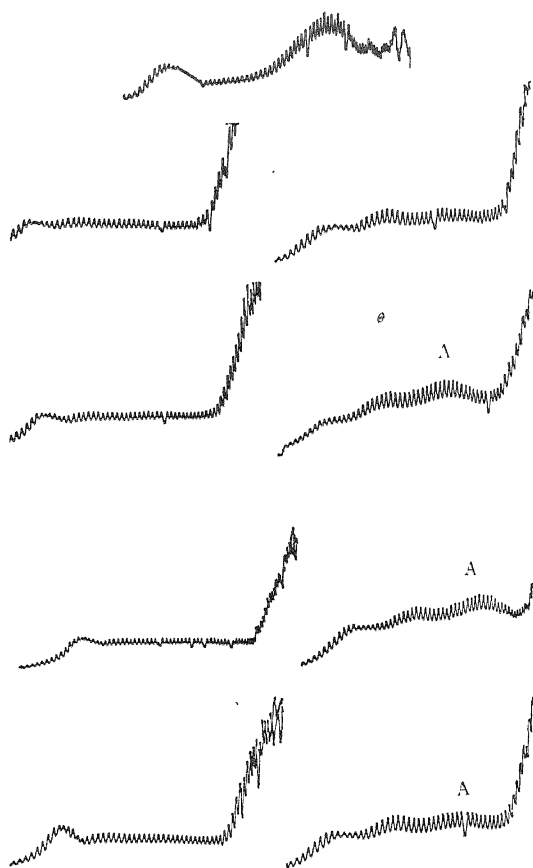


Fig. 1. The top curve is from cystine. The curves to the left are from heated milk samples, and to the right are the corresponding curves from unheated milk samples.

the lower voltage. The vertical distance between the two lines is a function of the difference in the concentration of the sulfhydryl groups.

The data in Table I give the calculated values of the sulfhydryl groups in the milk samples in terms of cysteine in the quantity of milk used for each curve—that is, 1 ml of milk diluted 1 to 25. They are also given in equivalents of cysteine-hydrochloride in 210 ml of milk, which is the approximate quantity of milk used with 300 g of flour for the mixing of a dough in the farinograph. The reactive organic sulfhydryl groups apparently varied appreciably with each raw-milk

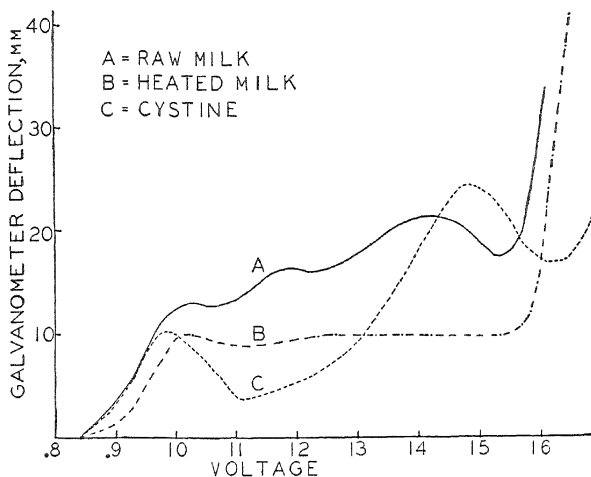


Fig. 2 Three typical curves showing the galvanometer deflection and the applied voltage.

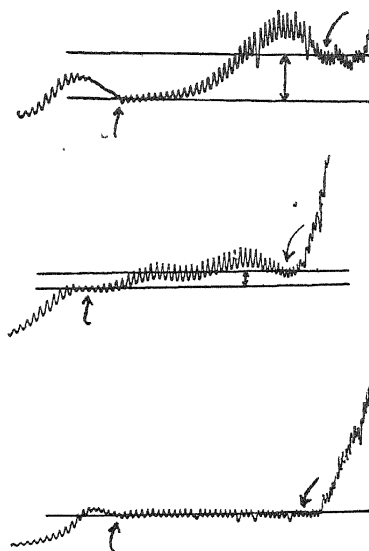


Fig. 3. This shows how the minimum just past the maximum was used for quantitative estimations. The top curve was obtained with a cystine solution of known concentration (final dilution $10^{-4}M$), the middle curve from unheated milk, and the lower curve from the same milk after a heat treatment.

TABLE I
CALCULATED QUANTITATIVE RELATIONSHIPS OF THE POLAROGRAPH CURVES

Milk used	Height of curve	Amount of reactive cysteine under the conditions of the polarograph test in milk used for test	Approximate equivalent of cysteine-hydrochloride in 210 ml milk
		mm mg	mg
(1 ml $10^{-4}M$ cysteine)	12	0.024	—
Boiled No. 1	0	*	*
Raw No. 1	4	0.008	52
Boiled No. 2	0	†	†
Raw No. 2	3	0.006	39
Boiled No. 3	0	*	†
Raw No. 3	5	.010	65
Boiled No. 4	0	*	*
Raw No. 4	4.5	.009	59
Average	—	—	52

* Quantities approaching 0

sample, but all contained a sufficient quantity to cause a degradation of the dough. Addition of 39 to 65 mg of cysteine-hydrochloride to a 300-g flour dough would produce a rapid degradation of the dough during the mixing process. The boiled milk had no measurable amount of sulfhydryl groups.

These studies seem to indicate the necessity of heating milk properly for improved baking quality, and that elimination of the dough-softening action is a process of oxidation or occlusion of exposed sulfhydryl groups present in the raw milk.

Farinograph Tests

The farinograph or other recording dough mixers are recognized as being useful in the study of the baking quality of milk. A poor-quality milk will tend to give a farinogram showing a rapid dough softening after the point of maximum plasticity.

Using a medium-strength flour with about 11% protein and 2% salt (flour basis) in each dough, a series of farinograms was made with fresh milk and the whey and curd separated from the same milk sample by means of both acid and rennet coagulation.

A fresh separated milk sample was allowed to sour by standing at room temperature for 30 hours, and another sample was rennet-coagulated by using sufficient rennet to coagulate the milk in one hour at room temperature. The curd and the whey were separated by centrifugation. The whey was then given a five-minute heat treatment at 97°–100°C, cooled to 30°C, and recombined with the curd, and this preparation used for a farinogram with the described flour. Other farinograms were made similarly using the fresh milk, the coagulated milk, and the milk heated to 97°–100°C for five minutes.

The data in Table II give the results of these tests. The farinograms were analyzed as to time to reach minimum mobility, the maximum mobility, and the summation value. The summation value is the total value of all the breakdown units of the curves from the peak to the 30-minute stage using the units at every even-numbered minute. The actual curves are reproduced in Figure 4.

It is evident that the factor responsible for the dough softening action in unheated milk is present primarily and probably entirely in the whey portion. The curd undoubtedly retained some adsorbed whey, which accounts for the slight difference between the curves from heated milk and heated whey. Souring or coagulation alone did not materially change the curves.

TABLE II

RESULTS OF FARINOGRAPH TESTS USING A STANDARD FLOUR AND VARIOUSLY TREATED MILK PREPARATIONS

Curve no.	Material	Heat treatment	Time of minimum mobility	Minimum mobility	Summation value
		$^{\circ}\text{C}$	<i>min</i>	<i>FU</i>	<i>FU</i>
1	Fresh separated milk	None	9.5	520	1350
2	Same milk rennet coagulated	None	11.0	495	885
3	Rennet coagulated milk, whey heated	97 $^{\circ}$ -100 $^{\circ}$ 5 min	13.0	485	510
4	Fresh separated milk	97 $^{\circ}$ -100 $^{\circ}$ 5 min	16.0	495	250
5	Fresh separated milk	None	10.5	520	1290
6	Soured milk, 30 hrs. room temp.	None	10.0	500	1105
7	Soured milk, whey heated	97 $^{\circ}$ -100 $^{\circ}$ 5 min	13.0	490	315
8	Fresh separated milk	97 $^{\circ}$ -100 $^{\circ}$ 5 min	17.0	490	105

Since the rennet whey gave the same results as the acid whey, it appears likely that calcium is not a factor involved. In the case of rennet coagulation, practically all of the calcium remains with the curd, but with acid-coagulated milk the calcium is found in the whey portion.

Several other tests including the use of hydrochloric-acid-coagulated milk also gave similar results, indicating that the dough-softening factor was present in the whey portion of the milk.

Discussion

The polarograms of raw milk have characteristics in common with polarograms of cysteine-cystine solutions. These characteristics disappear upon heat treatment. Considered as cysteine the quantity of such groups in raw unheated milk appeared more than sufficient to

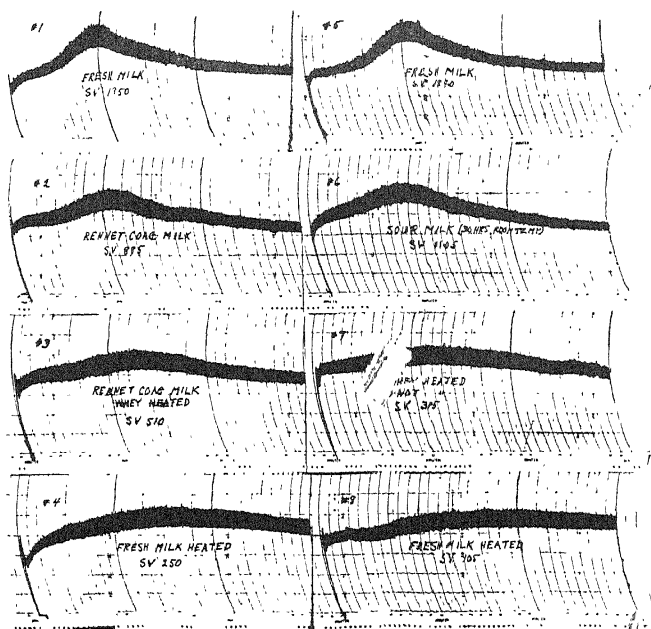


Fig. 4. Farinograms using fresh unheated milk (No. 1 and No. 5), coagulated milk (No. 2 and No. 6), coagulated milk in which the whey only was heat-treated (No. 3 and No. 7), and the fresh milk heat-treated (No. 4 and No. 8).

cause a softening action in wheat-flour doughs made with milk. The farinograph tests showed that the factor causing the dough softening was present in the whey portion of the milk. It is probably the lactalbumin which is involved, although the work of Sørensen and Sørensen (1939) indicates that the whey proteins are a complex mixture of several fractions. Computed from polarograms of the unheated milk samples the equivalent of 52 mg of cysteine hydrochloride in 210 ml of milk was present, which represents only about one-tenth of the sulfur in lactalbumin or about 15 hundredths of the total sulfur content of milk. Hence only a small portion of the total sulfur appears to be present as exposed or reactive groups in unheated milk under the conditions of this test.

While both the casein and the lactalbumin contain cystine sulfur, the concentration is generally reported to be much higher in the lactalbumin. Casein is present in milk in about ten times the amount of lactalbumin, yet it appears that the higher-molecular-weight casein proteins have few if any exposed or reactive sulfhydryl groups.

Sjögren and Svedberg (1930) after a study of milk with the ultra-

centrifuge concluded that lactalbumin as it occurs in raw milk is much more dispersed than any of the isolated products and that perhaps lactalbumin is not homogeneous. Their studies gave the molecular weights of 12,000 to 25,000 for the isolated lactalbumin, but they state that in the raw milk the bulk of the lactalbumin probably does not exceed a molecular weight of 1,000. Some authors (Hotchkiss, 1939) object to this rather low figure, but it is generally agreed that the molecular weight of lactalbumin is much lower than that of many other proteins. Ansbacher and Supplee (1934) and Supplee (1940) have shown that lactalbumin contains prosthetically bound lipid material in a fairly constant concentration of 7%, of which about 40% is cholesterol.

Some further experiments have shown that the dough-softening principle in unheated milk or whey dialyzes through Visking casing, as shown by farinograph tests only, but no direct tests for sulfhydryl groups were made. This indicates that a low-molecular-weight substance is involved. When the diffusate was heated a turbidity appeared indicating the presence of the heat-coagulable proteins.

While the experiments included in this report indicate that the heat treatment ordinarily given milk for the improvement of baking quality causes a decrease in groups reacting like cysteine, it is well known that denaturation or heating of egg albumin causes a marked increase in free sulfhydryl groups (Bull, 1938; Mirsky, 1938). However, a more severe heat treatment of milk or whey may cause a denaturation with a similar liberation of sulfhydryl groups. In fact Grewe and Holm (1928) and Skovholt and Bailey (1930) found that heating of milk at about 75° gave the best baking results, while higher temperatures or prolonged heating were less desirable.

Several workers (Gould and Sommer, 1939; Josephson and Doan, 1939) have observed that minute traces of hydrogen sulfide are formed when milk is heated and these cause the cooked flavor. Such small amounts of hydrogen sulfide probably would have no appreciable action in a milk dough and would perhaps be partially eliminated through aeration during the mixing process, but such quantities of hydrogen sulfide are still sufficient for organoleptic detection.

It appears that the following theory might account for the dough-softening action of unheated milk and the effect of the proper heat treatment: Fresh raw milk contains low-molecular-weight whey proteins high in sulfur, and these apparently are the proteins generally referred to as lactalbumin. These small protein molecules have some exposed or reactive sulfhydryl groups representing only a small part of the total sulfur. Upon proper heat treatment these proteins aggregate into particles of higher molecular weights. An oxidation of the sulfhydryl groups to the more stable disulfide linkage may take place,

or the sulfhydryl groups may be masked by occlusion. During this process, minute traces of sulfhydryl groups are released from the protein, forming free hydrogen sulfide, which may be of no material significance in terms of baking quality or dough-softening action but which causes the cooked flavor.

The heat treatment of milk necessary for the improvement of the baking quality appears to be analogous to the effect of the heat treatment of wheat. Sullivan, Howe, and Schmaltz (1937) showed that at least part of the beneficial effect of the heat treatment of wheat was the oxidation of the glutathione in the germ portion.

In this report the dough-softening factor in unheated milk has been discussed, but actually the baking quality of milk involves other factors also and particularly when evaporated or dried milk products are considered. Such other factors as absorption capacity, color, and flavor are also included in the general term of baking quality of milk products, but the dough-softening action of improperly heat-treated milk is one of primary importance in relation to baking quality.

Summary

It is known that a heat treatment is necessary to improve the baking quality of milk. Several samples of fresh unheated separated milk were subjected to polarographic analyses and each sample gave a current-voltage curve analogous to those obtained with cysteine-cystine solutions. When the same samples were heated for five minutes at 97°–100°C the polarograms no longer had this characteristic.

Quantitative estimations of the sulfhydryl groups in the raw-milk samples showed them to be present in amounts sufficient to cause a pronounced dough-softening action as compared to the action of cysteine hydrochloride.

After separating the curd and the whey of milk, it was shown by means of farinograms that the dough-softening factor in unheated fresh milk was present in the whey portion, and probably in the lactalbumin.

An hypothesis is advanced to explain the effect of heat treatment of raw milk in eliminating the dough-softening action. It is postulated that low-molecular-weight lactalbumin molecules with exposed sulfhydryl groups combine during the heat treatment to give protein aggregates of higher molecular weights. During this process the sulfhydryl groups are either oxidized to the more stable disulfide linkage, or masked by occlusion.

Acknowledgment

The advice of Dr. R. A. Gortner and Dr. Eric Roberts in regard to the polarographic work is most earnestly appreciated.

Literature Cited

- Ansbacher, S., and Supplee, G. C.
1934 The cholesterol content and the antirachitic activation of milk constituents. *J. Biol. Chem.* **105**: 391-404.
- Baernstein, H. D.
1936 The sulfur distribution in proteins. II. *J. Biol. Chem.* **115**: 33-36.
- Balls, A. K., and Hale, W. S.
1936 Further studies on the activity of proteinase in flour. *Cereal Chem.* **13**: 656-664.
- Brdicka, R.
1933 Polarographic studies with the dropping mercury electrode. *Coll. Czechoslov. Chem. Com.* **5**: 148-164, 238-253.
- Bull, H.
1938 Protein denaturation. Symposia on quantitative biology. Cold Spring Harbor **6**: 140-144.
- Ford, W. P., and Maiden, A. M.
1938 Effects on dough of glutathione and papain. *J. Soc. Chem. Ind.* **57**: 278-281.
- Frelich, J., and Frey, C. N.
1939 Dough oxidation and mixing studies. III. *Cereal Chem.* **16**: 503-512.
- Gould, I. A., Jr., and Sommer, H. H.
1939 Effect of heat on milk with special reference to the cooked flavor. *Tech. Bul.* 164, Mich. State College, East Lansing.
- Grewe, E., and Holm, G. E.
1928 Effect of variation in the method of manufacture on the baking quality of dry skimmilk. *Cereal Chem.* **5**: 461-469.
- Hotchkiss, R. D.
1939 The determination of peptide bonds in crystalline lactoglobulin. *J. Biol. Chem.* **131**: 387-398.
- Josephson, D. V., and Doan, F. J.
1939 Cooked flavor in milk. Its source and significance. *Milk Dealer* **29**: (No. 2) 36, 54-62.
- Mirsky, A. E.
1938 Protein denaturation. Symposia on quantitative biology, Cold Spring Harbor **6**: 150-153.
- Mohr, W., and Wellm, J.
1936 Use of the polarographic method in the investigation of solutions containing milk proteins. *Milchw. Forsch.* **18**: 123-130.
- Sjögren, B., and Svedberg, T.
1930 The molecular weight of lactalbumin. *J. Am. Chem. Soc.* **52**: 3650-3654.
- Skovholt, O., and Bailey, C. H.
1930 Report to the American Dry Milk Institute. September 25, 1930.
- Smith, E. R., and Rodden, C. J.
1939 Effects of methionine, djenkolic acid, and benzylcysteine on the estimation of cystine by the dropping mercury cathode. *Nat. Bur. Standards J. Res.* **22**: 669-672.
- Sørensen, S. P. L., and Sørensen, Margrethe
1939 The proteins in whey. *Compt. rend. trav. lab. Carlsberg (Ser. Chim.)* **23**: 55-99.
- Sullivan, B., Howe, M., and Schmaltz, F.
1937 An explanation of the effect of heat treatment on wheat germ. *Cereal Chem.* **14**: 489-495.
- Supplee, G. C.
1940 Whey as a source of vitamins and vitamin products. *Ind. Eng. Chem.* **32**: 238-240.

A STATISTICAL STUDY OF THE DATA OF SUB-COMMITTEES ON TESTING BISCUIT AND CRACKER FLOURS

CHARLES A. TARNUTZER¹

1109 East Richwoods, Peoria, Illinois

(Received for publication June 16, 1941)

The subcommittee of 1938-39 (Simmons, 1940) recommended:

1. That further work be done to build up a greater volume of data correlating the laboratory evaluation of cracker flours with their actual shop performance.
2. That further study be made on the scoring of crackers in order to obtain a closer agreement among collaborators.
3. That a statistical study be made of all pertinent data accumulated by the committees in the last three years, this to be done in order that more practical conclusions may be reached.

The first tool needed to correlate more closely the laboratory evaluation of cracker flours with their actual shop performance is a scoring method for crackers which will enable collaborators to agree as to which is the best cracker. The method of scoring should enable each collaborator to reach a definite numerical decision. Only then will it be possible to use the analytical data, the viscosity, and the baking tests to pick out a good sponge flour or a good dough flour.

Scoring Crackers

The present method of scoring makes it very difficult for a collaborator to recheck his decisions. It provides no mathematical score or measurable characteristics which can be scored in degrees of perfection.

Looking at Table V (Simmons 1940), one concludes that the first bakes of crackers P1, P2, P3, and P4 were so much alike that the collaborators were unable to agree as to just what position each cracker should be given. The second bake appeared to be slightly easier to score, but only P4 received the same rank as in the first bake. Surely the two bakes did not differ as much as the results show. Statistical correlation shows the marked inability of each collaborator to duplicate his results.

The scores for cracker P1, bake 1, were correlated with the scores for cracker P1, bake 2, using the results of eight collaborators. According to Wallace and Snedecor (1931) the value of the coefficient would have to be at least $+ .707$ to be significant, and to be highly significant the coefficient should be $+ .834$ or higher. The correlation

¹ Present address: Hiram Walker & Sons, Inc., Peoria, Illinois.

coefficient for each cracker baked from Pacific coast flours shows that only the results given for cracker P3 are significant (Table I).

TABLE I
CORRELATION OF BAKES OF PACIFIC COAST FLOURS

Crackers	Coefficient of correlation
P1	+.10
P2	+.43
P3	+.74
P4	+.09

The same correlation study was made for crackers A, B, C, and D baked from midwest flours. Bakes 1 and 3 were used for this study; bake 2 was discarded because the crackers in this bake had an unusually high ash content (Table II).

TABLE II
CORRELATION OF BAKES OF MIDWEST FLOURS

Crackers	Coefficient of correlation
A	+.43
B	+.28
C	+.27
D	+.56

None of the scores for the midwest flours is significant.

In bakes 1 and 2 for the four crackers examined by the subcommittee of 1937-38 (Brown, 1939), the collaborators had much difficulty in correlating their results; in many cases negative correlation was shown (Table III).

TABLE III
CORRELATION OF CRACKERS BAKED IN 1937-38

Crackers	Coefficient of correlation
1	-.04
2	-.65
3	+.05
4	+.07

The method of scoring outlined by the Committee in their report of 1936-37 (Reiman, 1938) gives much importance to the shortness of the cracker. Forty out of the possible 100 points for a perfect cracker stress shortness in some degree, with 10 points for flakiness, 15 points for crispness, and 15 points for tenderness. If each collaborator is able

to score tenderness on this basis we should expect good correlation between the rank of the cracker and its shortometer rank (Table IV).

The correlation between rank and shortometer reading for midwest crackers, bakes 1 and 3, was + .41. The correlation between rank and shortometer reading for crackers in the 1937-38 report (bake 1, bake 2, and bake 3) was - .20.

Granting that an instrument such as a shortometer will give a correct numerical evaluation for shortness; and granting that flakiness, crispness, and tenderness scored by individual collaborators should reflect 40% of a total score for a cracker, we should expect—if the

TABLE IV
CORRELATION BETWEEN RANK AND SHORTOMETER READING
FOR P CRACKERS, BAKE 1 AND BAKE 2

Crackers	Rank		Shortometer		
	ΣA	ΣA^2	ΣX	ΣX^2	ΣAX
Bake 1					
P1	1	1	86.6	7499.6	86.6
P2	2	4	83.7	7005.7	177.4
P3	3	9	87.3	7621.3	261.9
P4	4	16	87.6	7673.8	350.4
Bake 2					
P1	3	9	83.7	7005.7	251.1
P2	1	1	67.5	4556.3	67.5
P3	2	4	82.7	6842.3	165.4
P4	4	16	71.9	5169.6	287.6
	20	60	651.0	53374.3	1647.9

$$MA = 2.5$$

$$MX = 81.3$$

$$r = \frac{\Sigma AX - (\Sigma A)MX}{\sqrt{\Sigma A^2 - (\Sigma A)MA} \sqrt{\Sigma X^2 - (\Sigma X)MX}} = 0.33$$

collaborator is as accurate as the shortometer—a correlation coefficient of at least + .707. None of the above correlation coefficients is significant. The judging of the crackers of 1937-38 showed a negative correlation. The method of scoring used is obviously unreliable, for it depends upon the appraisals of collaborators whose judgments did not agree with the shortometer values.

The collaborator is unable to check himself on a value which involves 40% of the rating of an individual cracker. In addition 20% of the total rating represents personal reaction of the collaborator as to the flavor of the cracker. Flavor is a most delicate and difficult entity to characterize and systematically record.

The method of scoring allows 3% for symmetry of edge, 3% for shape, and 5% for salt; none of these has any relation to flours but to the fermentation and machining of the doughs. Therefore 71% of the total score is very difficult to check; it is no wonder that correlation between collaborators and bakes was not significant.

Correlation between Moisture and Shortometer Reading

As a cracker takes up moisture it becomes more soggy, less crisp, and less tender; hence one would expect the shortometer reading to be higher for a cracker with more moisture. In the 1938-39 report (Simmons, 1940), Table VII gives the moisture content and the shortometer values. Using the values in these two columns one obtains a correlation coefficient of $+ .203$. To show a definite correlation between moisture and shortometer values the coefficient should be at least $+ .444$.

Using the same type of data from the 1937-38 report (Brown, 1939) one obtains a correlation coefficient of $+ .79$. To show a definite correlation between moisture and shortometer values the coefficient should be at least $+ .576$, and the correlation is highly significant if it is $+ .708$ or higher.

The moisture content of the cracker actually has some effect on the shortometer reading, but other factors are also important, as shown by the fact that the correlation for data from 1938-39 report is not significant.

Chemical and Physical Analysis of Crackers

Because all of the crackers should have been baked by the same formula, one would expect little difference in individual cracker analytical results. There is little difference in fat content if the results are figured on a dry basis. One would expect only small differences in flour protein and ash content, but for some unknown reason bake 4 (1938-39) gave crackers with exceptionally high ash and low protein content. Because of the abnormality in bake 4 none of its results were used in this study.

It is impossible to find any definite correlation between the score of a cracker and its protein, ash, or fat content. The crackers baked from the Pacific coast flours are the lighter crackers. The shortometer readings for the Pacific group are higher than those for the midwest group, but this can be expected because of the 1.5% lower fat content. It appears that chemical analyses of crackers, where all are baked by one formula, have little value for classifying flours used.

The count per pound and the average thickness of 10 crackers shows oven spring; and where flours of different strengths are used it is certain

TABLE V
CHEMICAL ANALYSIS OF CRACKERS (1938-39)

Cracker number	Protein, $N \times 6.25$	Ash	Fat	Shortometer value	Count per pound
	%	%	%		
P1	10.16	2.42	12.19	85.1	120
P2	10.20	3.03	12.40	75.6	122
P3	10.75	2.26	12.50	85.0	120
P4	10.80	2.80	12.53	79.8	120
A	9.18	2.76	14.43	76.3	111
B	9.03	2.53	14.33	75.3	116
C	9.07	2.67	14.09	81.2	111
D	8.88	2.95	14.01	70.0	112

that such data would be valuable. One other variable, the area, should be included in the physical analyses—namely, the flat area of four crackers. Such a measurement would reflect oven spread, which is presumably a measurement of strength.

Do Analytical Data Help to Indicate a Use for Flour?

Definite standings or ratings expressed as numbers are necessary to study correlation. For this reason a numerical rating is given in the column headed "Used For," Table I, 1938-39 report.

Weak dough.....	10
Medium dough.....	20
Strong dough.....	30
Weak sponge.....	40
Medium sponge.....	50
Strong sponge.....	60

In all the following correlation studies, the coefficient of correlation must be at least $+ .707$; and to be highly significant it must be $+ .834$ or higher.

Using the ash analyses as one variable and the numerical ratings from column 5, Table I, 1938-39 report, as the other variable, the coefficient of correlation was $+ .33$. Ash analyses are not useful for indicating the utility of flours.

With pH values as one variable and the numerical ratings as the other, the coefficient is $+ .31$; and thus the hydrogen ion concentration of the flour has no value in suggesting specific utility.

A correlation coefficient of $+ .793$ was found when the protein values and the numerical ratings for the last four subcommittee reports were used. This figure is highly significant. The 1938-39 study gave a coefficient of $+ .92$ for the Pacific flours, and a significant figure of $+ .98$ was obtained for the midwest flours.

The protein content of a flour as a measure of its utility—if Column 5 of Table I, 1938-39 report is indicative of bake-shop use—is sta-

tistically important. If the designations in column 5 are merely millers' opinions, and do not reflect actual shop experience, it is evident that the opinions are based largely upon protein analysis.

Table II, 1938-39 report, gives average viscosity determinations using the one-hour and no-time methods. With one-hour values as one variable and the no-time values as the other, it was possible to get a correlation coefficient of 1.00. This is a perfect correlation, and it shows that the same factors present in the no-time method are equally important in the one-hour method.

Do Investigators Use the Viscosity Test to Classify Flours?

Table VI shows the numerical ratings given to data taken from Table III, 1938-39 report. To prove further that viscosity readings are very important in the final choice of the use of a flour, the average ratings of Table VI were correlated with the actual viscosity readings. The flours of the 1938-39 study, numbers 9 to 17, gave a correlation coefficient of $+ .92$. Flours of the 1937-38 study gave a similar significant coefficient.

The viscosity test is very important to help classify flours, and all collaborators are using the viscosity readings to classify flours for use in the bakeshop.

TABLE VI
CLASSIFICATION ON BASIS OF ANALYTICAL AND VISCOSITY TESTS

Collaborator	Flour number							
	9	10	11	12	13	14	15	16
1	50	50	20	20	50	50	20	20
2	50	50	—	20	50	50	35	—
3	50	50	—	20	50	50	20	20
4	50	50	10	20	50	50	20	20
5	50	60	—	—	40	20	—	10
6	50	50	—	20	50	50	20	—
7	50	50	20	20	50	50	20	10
8	50	50	—	20	50	50	30	10
Average rating	50	51	17	20	49	46	24	15

Do Investigators Use the Baking Test to Classify Flours?

Classification on the basis of baking tests compares very closely with that based on viscosity tests. The 1938-39 report shows that flour 15, and the 1937-38 report shows that flour 8, are exceptions to the statement above. Unfortunately crackers were not baked from formulas using the two questionable flours as sponge flours. Every

flour to be tested should be tried as a sponge flour, for it is a well known fact that dough flours have little effect upon the finished cracker. It is interesting to note that crackers baked with flour 14 as the sponge flour and flour 15 as the dough flour gave a very inferior cracker. Perhaps the baking test will serve as a means of identifying the occasional flour that causes trouble in the bakeshop.

Table VII, showing a correlation coefficient of $+ .75$, indicates that the baking test is serviceable in evaluating the use of flours, being almost equivalent to the viscosity test. Should the data for the abnormal flours 15 and 8 have been excluded, better correlation between baking test and viscosity test would have resulted.

TABLE VII

CORRELATION BETWEEN THE RATINGS BASED ON VISCOSITY AND BAKING TESTS

Year and flour	Evaluation by viscosity		Evaluation by baking		
	ΣA	ΣA^2	ΣX	ΣX^2	ΣAX
1938					
5	26	676	21	441	546
6	25	625	20	400	500
7	50	2500	39	1521	1950
8	18	324	40	1600	720
1939					
9	50	2500	46	2116	2300
10	51	2601	51	2601	2601
11	17	289	18	324	306
12	20	400	20	400	400
13	49	2401	49	2401	2401
14	46	2116	50	2500	2300
15	24	576	48	2304	1152
16	15	225	13	169	195
	391	15233	415	16777	15371

$$MA = 32.6 \quad MX = 34.6 \quad r = +.75$$

Summary

There is definite need of a scoring method for crackers which will enable collaborators to agree as to which is the best product.

The moisture content of a cracker has some effect on the short-ometer reading, but other factors are also important.

Protein content and viscosity readings are statistically important as a measure of flour utility.

Classification of flours on the basis of baking tests agrees very closely with classification based on viscosity. The baking test may give additional means for the identification of flours that are likely to cause trouble in the bakeshop.

Literature Cited

- Brown, Pearl
1939 Report of the 1937-38 subcommittee on testing biscuit and cracker flours. *Cereal Chem.* **16**: 130-136.
- Reiman, W.
1938 Report of the 1936-37 committee on testing biscuit and cracker flours. *Cereal Chem.* **15**: 37.
- Simmons, H. M.
1940 Report of the 1938-39 subcommittee on testing biscuit and cracker flours. *Cereal Chem.* **17**: 250-256.
- Wallace, H. A., and Snedecor, G. W.
1931 Correlation and machine calculation. Official Publication of Iowa State College 20, No. 4.

THE RELATION BETWEEN THE VITAMIN-A-ACTIVE
CAROTENOIDS IN CORN AND THE NUMBER
OF GENES FOR YELLOW COLOR

~ A. R. KEMMERER, G. S. FRAPS, and P. C. MANGELSDORF

Division of Chemistry, Texas Agricultural Experiment Station,
College Station, Texas

(Received for publication January 13, 1942)

Yellow corn, unlike most plant materials, contains a large part of its vitamin-A-active pigments in the form of cryptoxanthin (Buxton, 1939; Fraps and Kemmerer, 1941; Kuhn and Grundmann, 1934) which, according to Kuhn and Grundmann (1933), has approximately one-half the vitamin A potency of beta-carotene.

Mangelsdorf and Fraps (1931) reported a linear relationship between the number of genes for yellow pigmentation in yellow corn, and in its hybrids with white, and the biological vitamin A potency of the corn as measured by the Sherman-Munsell method. Hauge (1930) and Hauge and Trost (1930) also have reported that there is a high degree of association between the number of genes for yellow color and the vitamin A potency. Johnson and Miller (1938, 1939) found a close relationship between the total carotenoid in yellow corn and the genetic constitution. Randolph and Hand (1938, 1940) found that doubling the number of chromosomes in yellow corn increased the total carotenoid pigments by approximately 40%. However, doubling the chromosomes also increased the volume of the endosperm cells, so that the actual amount of carotenoids elaborated per gene was approximately 2.5 times as great in the tetraploid as in the diploid. Randolph and Hand also found that the vitamin-A-active fractions, including both beta-carotene and cryptoxanthin, increased in approximately the same degree.

Recent investigations by Fraps and Kemmerer (1941) have shown that yellow corn contains, in addition to alpha-carotene, beta-carotene,

and cryptoxanthin, a carotene termed K-carotene. Methods for determining the quantities of these carotenes in yellow corn were developed, and it was shown that K-carotene, like alpha-carotene and cryptoxanthin, has only approximately one-half the vitamin A potency of beta-carotene.

With newer and improved methods of analysis available, it seemed desirable to ascertain the relationship between number of genes and amounts of carotenoid pigments contained in the crude carotene.

As in the previous work (Mangelsdorf and Fraps, 1931) appropriate pollinations were made to produce four classes of seeds, roughly described as white, pale yellow, dilute yellow, and deep yellow. In the case of the factor for yellow pigmentation, the cells of the endosperm have 0, 1, 2, or 3 genes for the yellow pigment, with the corresponding factorial composition, yyy , yyY , yYY , or YYY . Four varieties of white corn, Paymaster, Surecopper, Mexican June, and Thomas, and four varieties of yellow corn, Yellow Paymaster, Yellow Surecopper, Golden June, and Golden Thomas, were used. All pollinations were made between the yellow and white strains of the same variety. In the case of the last three, the yellow strains were developed from the white by hybridization and repeated backcrossing and, with the exception of the genes for color, were approximately isogenic with the original white varieties. The procedure followed in this experiment has thus eliminated variations in carotene content resulting from differences between white and yellow varieties in the size, shape, and texture of the grain.

Experimental

The samples of corn were finely ground, refluxed with alcoholic potassium hydroxide, and the crude carotene fraction obtained as in the A. O. A. C. method for carotene (A. O. A. C., 1940). This crude carotene fraction has been shown to contain all the vitamin-A-active constituents of yellow corn (Fraps and Kemmerer, 1941). The pigments in the crude carotene fraction were separated and identified by running the solutions through a column of magnesium oxide by a method reported also by Fraps and Kemmerer.

The results of this study are shown in Table I. Although there are some variations, the data show as has been reported in previous work (Mangelsdorf and Fraps, 1931) that the number of genes for yellow color in corn directly affects the amount of carotenoid pigments and the vitamin A potency. The corns of the genic constitution YYY contained on the average 6.4 ppm of crude carotene, the corn of YYy constitution 4.2, and the corns of Yyy constitution 1.8. The corn of yyy constitution contained only 0.1 ppm of crude carotene. The data

in this table also show that the number of genes for yellow color had no significant influence on the average percentage of alpha, beta, and K carotene and cryptoxanthin comprising the crude carotene.

There were some variations between the individual samples in the percentages of the carotenoids in the crude carotene. For example, all of the samples in which the genes for yellow color were derived from

TABLE I
EFFECT OF THE NUMBER OF GENES FOR YELLOW COLOR ON THE VITAMIN-A-ACTIVE PIGMENTS OF YELLOW CORN

Variety of corn	Crude carotene	Constituents of crude carotene				
		Alpha carotene	Beta carotene	K carotene	Cryptoxanthin total	Impurity
YY' group	ppm	%	%	%	%	%
Yellow Paymaster	3.9	4.8	33.2	6.4	52.6	0
Yellow Surecopper	6.6	3.5	36.0	5.5	52.2	2.9
Golden June	7.1	4.2	22.1	3.6	66.7	3.6
Golden Thomas	7.8	3.8	37.8	3.9	51.2	1.8
Average (4)	6.4	4.1	32.3	4.9	55.7	2.1
YYy Group						
Yellow Paymaster X	3.7	4.3	36.4	5.2	53.0	1.7
White Paymaster						
Yellow Surecopper X	3.6	6.6	27.6	3.3	58.6	3.8
White Surecopper						
Golden June X	3.9	8.1	18.3	4.1	65.6	4.1
White June						
Golden Thomas X	5.5	4.4	32.0	3.3	58.2	2.6
White Thomas						
Average (4)	4.2	5.9	28.6	4.0	58.9	3.1
Yyy Group						
White Paymaster	0.9	7.3	27.7	8.0	54.5	2.7
X Yellow Paymaster						
White Surecopper	1.1	—	—	—	49.9	6.4
X Yellow Surecopper						
White June	2.5	4.0	19.8	5.9	57.3	12.9
X Golden June						
White Thomas	2.5	3.3	37.5	4.2	50.8	5.0
X Golden Thomas						
Average	1.8(3)	4.9(3)	28.3(3)	6.0(4)	53.1(4)	6.8

Golden June were consistently higher in cryptoxanthin than were the remaining three samples. Fraps and Kemmerer (1941) have previously shown that corn may be divided into two rather distinct groups with respect to the constituents of the crude carotene. Whether or not variations of this kind were a consequence of differences in behavior of the Y gene on different genetical backgrounds, or the effect of various

modifying factors, was impossible to determine from this experiment. Nevertheless, it is clear that there is an almost perfect linear relationship between the genic constitution of the endosperm and the amount of crude carotene, and that this relationship holds approximately for each of the separate constituents of crude carotene.

Conclusion

The number of genes of yellow color in corn directly affects the amount of vitamin-A-active carotenoid pigment, but does not affect appreciably the relative proportions of the different constituents, alpha, beta, and K carotene and cryptoxanthin.

Literature Cited

- Association of Official Agricultural Chemists
1940 Methods of analysis (5th ed.). A. O. A. C. Washington, D. C.
- Buxton, L. O.
1939 Carotenoids in yellow corn. *Ind. Eng. Chem. (Anal. Ed.)* **11**: 128-129.
- Fraps, G. S., and Kemmerer, A. R.
1941 Determination of carotene and cryptoxanthin in yellow corn. *Ind. Eng. Chem. (Anal. Ed.)* **13**: 806-809.
- Hauge, S. M.
1930 An inheritance study of the distribution of vitamin A in maize. II. Vitamin A in hybrid red maize. *J. Biol. Chem.* **86**: 161-165.
- and Trost, J. F.
1930 An inheritance study of the distribution of vitamin A in maize. III. Vitamin A content in relation to yellow endosperm. *J. Biol. Chem.* **86**: 167-172.
- Johnson, I. J., and Miller, E. S.
1938 Variation in carotenoid pigment concentration among inbred and cross-bred strains of corn. *Cereal Chem.* **15**: 345-350.
- 1939 Immediate effect of cross pollinations on the carotenoid pigment in the endosperms of maize. *Cereal Chem.* **16**: 88-92.
- Kuhn, R., and Grundmann, C.
1933 The growth vitamin. V. Cryptoxanthin, a xanthophyll of the formula $C_{40}H_{56}O$. *Ber.* **66B**: 1746-1750.
- 1934 The growth vitamin. VI. Cryptoxanthin from yellow corn. *Ber.* **67B**: 593-595.
- Mangelsdorf, P. C., and Fraps, G. S.
1931 A direct quantitative relationship between vitamin A in corn and the number of genes for yellow pigmentation. *Science* **73**: 241-242.
- Randolph, L. F., and Hand, D. B.
1938 Increase in vitamin A activity of corn caused by doubling the number of chromosomes. *Science* **87**: 442-443.
- 1940 Relation between carotenoid content and the number of genes per cell in diploid and tetraploid corn. *J. Agr. Res.* **60**: 51-64.

THE THIAMIN CONTENT OF WHEAT FLOUR MILLED BY THE STONE MILLING PROCESS

ALFRED S. SCHULTZ, LAWRENCE ATKIN, and CHARLES N. FREY

The Fleischmann Laboratories, Standard Brands, Incorporated, New York

(Read at the Annual Meeting, May 1941)

The modern roller mill, now almost universally employed for grinding wheat, has very little in common with the old stone mill. The roller mill grinds the wheat by a series of gradual reductions between carefully constructed and accurately spaced rolls; at no time is the wheat berry crushed or subjected to intense friction as it was in the mill-stone process. The difference in treatment has undoubtedly resulted in a different end product.

The introduction of roller mills occurred at a time when vitamins were quite unknown, and consequently we have no direct knowledge of the effect of the new process on the vitamin content of the flours then being produced. Recently Mitchener (1941), Miller (1941), and Rich (1941) have considered the changes which have occurred in the vitamin B₁ content of white flour produced by the old and new processes. Each concludes, reasoning by analogy, that the white flour produced one hundred years ago contained no more thiamin than the modern product.

According to these authors the ancients produced fine "white" flour equal to our modern flour. The evidence for this assumption is based on records which show that some of these old mills obtained a flour of only 55% to 70% extraction which they described as "white flour" or "superfine flour," etc. The analogy is completed by noting that the modern miller obtains the same low extraction and also calls his product "fine white flour." Obviously the adjective "white" as applied to flour was and still is a purely relative term.

Baker, Wright, and Drummond (1937) and Drummond and Wilbraham (1939) conclude that stone-ground white flour contained notably more thiamin than patent flour. Their contention is supported by analysis of stone-ground flour made in an English mill. Wheat containing 6.3 μg of thiamin per gram gave a flour containing 4.95 μg per gram. These authors emphasize the removal of germ by the roller mill but, as Schultz, Atkin, and Frey (1939) have shown, germ contains hardly more than 10% of the wheat thiamin. It seems more probable that stone-ground flour is richer in thiamin than patent flour because it retains a significant proportion of the aleurone layer of the wheat berry, which, if we may judge from the thiamin content of the

shorts of a modern mill stream, may contain more than 30% of the wheat thiamin.

Whatever the explanation of the thiamin distribution may be, it remains to be determined whether stone-ground white flours were relatively rich in thiamin or whether they were no better than modern patent flour, as Mitchener, Miller, and Rich claim. We have been fortunate in obtaining the cooperation of the managers of two American flour mills operating as closely as possible to the methods of a century ago. The mill streams of these mills were assayed by the fermentation method embodying the latest modification, sulfite cleavage (Schultz, Atkin, and Frey, 1942). The results are shown in Table I.

TABLE I
THIAMIN CONTENT OF STONE-MILL PRODUCTS¹

Fraction	Mill G, thiamin	Extraction	Mill P, thiamin (August)	Extraction	Mill P, thiamin (September)	Extraction
	$\mu\text{g/g}$	%	$\mu\text{g/g}$	%	$\mu\text{g/g}$	%
Bran	5.82	—	9.8	—	9.18	—
Middlings	6.27	—	4.46	—	6.0	—
Flour	4.04	54	4.74	55	4.52	62
Wheat	5.2	—	4.67	—	5.6	—

¹ The results of further work indicate that the high thiamin content of stone-ground flour is accompanied by proportionately high concentrations of other vitamins of the B complex as well as of ash.

The flours of the three millings represent 54%, 55%, and 62% extraction, respectively, which compares favorably with the low extractions obtained in milling modern patent flours. Mill G produced a flour containing 4.04 μg of thiamin per gram, or 78% as much as the original wheat. Mill P in August produced a flour slightly richer than the original wheat, and in September produced a flour containing 4.52 μg of thiamin per gram, representing 80% of the original wheat concentration.

The stone-milled white flours were fine products, somewhat creamier in color than modern patent flour. When baked into bread they could not stand color comparison with modern white bread but each gave bread that was far whiter than whole-wheat bread and it required no stretch of the imagination to believe that they would have been called "fine white bread" a hundred years ago. The comparisons mentioned were graphically displayed on Kodachrome transparencies but such photographs are not suitable for reproduction.

It must be concluded, therefore, that stone-ground white flour made today in the best-remembered fashion of past centuries contained a concentration of thiamin roughly equivalent to the whole grain.

This does not mean that all of the thiamin of the wheat berry was consumed directly by man for, as Table I shows, the offal or feed by-products were equal in thiamin content to the flour. Today the feed produced is far richer in thiamin than the original wheat.

The per-capita consumption of thiamin must have been far greater in the days of the stone mill than today because, as we are informed, the per-capita consumption of bread was higher and the bread that was eaten was notably richer in thiamin.

Summary

White flours produced at the present time in two stone mills operating in the manner of the past century were examined for thiamin content and found to contain thiamin in approximately the same concentration as the original wheat.

Literature Cited

- Baker, A. Z., Wright, M. D., and Drummond, J. C.
1937 The nutritive value of bread. *J. Soc. Chem. Ind. (Trans.)* **56**: 191T.
- Drummond, J. C., and Wilbraham, Anne
1939 *The Englishman's food*. Jonathan Cape, London.
- Miller, E. S.
1941 "Refinement" and the human race. *Northwestern Miller, Milling Production Section* **205** (No. 1): 3a.
- Mitchener, C. K.
1941 The grist Great-Granddad ground. *Northwestern Miller* **205** (No. 8): 10.
- Rich, C. E.
1941 Vitamin deficiencies and flour milling. *Chem. and Ind.* **60**: 611.
- Schultz, A. S., Atkin, L., and Frey, C. N.
1939 The vitamin B₁ content of wheat, flour, and bread. *Cereal Chem.* **16**: 643-648.
- 1942 The determination of vitamin B₁ by the yeast fermentation method. *Improvements related to the use of sulfite cleavage and a new fermentometer*. *Ind. Eng. Chem. (Anal. Ed.)* **14**: 35-39.

THE STABILITY OF VITAMIN B₁ IN THE MANUFACTURE OF BREAD

ALFRED S. SCHULTZ, LAWRENCE ATKIN, and CHARLES N. FREY

The Fleischmann Laboratories, Standard Brands Incorporated, New York, N. Y.

(Received for publication January 19, 1942)

The question of the stability of vitamin B₁ in the manufacture of bread gains increased importance as a result of the present national program for the enrichment of white bread. It is desirable to determine as far as possible the extent of vitamin B₁ loss in normal bakery practice so that the bread produced will meet whatever claims are made for it or whatever minimum standards may be set up. It is also desirable from the standpoint of economy to establish the conditions which result in minimum vitamin loss.

Recent reports (Hoffman, Schweitzer, and Dalby, 1940; Food and Drug Administration, Bread Hearings, 1941; Dawson and Martin, 1941) have indicated that the loss in thiamin during baking may vary from 5% to 30%. Part of the data of the present communication were presented at the bread hearings.

There are several factors which might conceivably affect the stability of thiamin during baking: the nature of the thiamin source, the time and temperature of baking, acidity, and the composition of the dough. We have found that the source of thiamin is without any significant effect; vitamin breads made with whole-wheat flour, white flour plus high-vitamin yeast, and white flour plus synthetic-thiamin all showed substantially the same destruction of thiamin when baked at the same temperature and for the same length of time. The time and temperature of baking appeared to have the greatest influence on the destruction of thiamin under our conditions and therefore several series of loaves were baked for different lengths of time but all at the same oven temperature.

Methods

The determination of thiamin was made by the yeast fermentation method, which included the sulfite modification (Schultz, Atkin, and Frey, 1942). In this method the thiamin content of the unknown is determined by the difference between two measurements of fermentation activity, one measurement before and one after treatment of the sample with sulfite. The first measurement determines the total fermentation stimulating substances (FSS) and the second determines the residual stimulators after the thiamin has been specifically inactivated by sulfite. The residual activity is called the "sulfite blank." The

thiamin content of the sample is represented by the difference between FSS and sulfite blank.

The sulfite blank reflects the presence of interfering aminopyrimidine fermentation activity, which is unaffected by sulfite treatment (Schultz, Atkin, Frey, and Williams, 1941). It has previously been observed and is also demonstrated in the present communication that the sulfite blank of a material is increased when its thiamin content is lowered as a consequence of excessive heat or alkalinity. The appearance of additional sulfite blank under these conditions is interpreted as being due to the formation of aminopyrimidines from thiamin, by a process of hydrolytic cleavage. The appearance of an unusually large sulfite blank is, therefore, an indication that thiamin has been destroyed by heat or alkali. The sulfite blank does not increase in exact proportion to the thiamin which has been destroyed, but one observes from 30% to 50% yield; *i.e.*, if 1.0 μ g of thiamin has been destroyed the sulfite blank is increased by 0.3 to 0.5 μ g.

Experimental

If the various ingredients of the dough batch are separately analyzed and a summation of these values considered as the vitamin input, it is possible that several small errors will be added together to give a result difficult to interpret. This is particularly true when one of the ingredients is patent flour, which is so low in thiamin content that most methods are least accurate with this material. It was preferred, therefore, to analyze the dough batch after mixing.

The laboratory bakes (Tables I to VI inclusive) were based on the straight dough process with the following formula (percentages expressed on the weight of flour): 2% salt, 5% cane sugar, 1% dry malt, 6% milk solids, $\frac{1}{3}$ % Arkady, and 2% yeast. For each experiment a dough batch containing 3,000 g of flour was mixed. This represented enough dough for 10 one-pound loaves of bread. The whole dough mass was weighed just before molding and one-tenth of the weight was scaled off for each of eight bakes. At the same time two weighed aliquots of the dough were taken for analysis. These were immediately macerated with water in a Waring Blendor, acidified, and heated at 100°C for 20 minutes. Two loaves of each of the doughs were baked for each of the following periods: 10, 20, 30, and 40 minutes—at 410°F in a gas-fired oven in which the loaves are rotated in a vertical plane.

The loaves were cooled for one hour at room temperature and then weighed. One loaf of each pair was prepared for total thiamin analysis by maceration in water, acidification, and heating at 100°C. The other loaf was dissected into three approximately equal portions: the crust with adhering crumb, the innermost crumb, and the intermediate

zone. Because of differences in moisture content it was necessary to air-dry these fractions and then determine the solids content of the air-dry samples. The thiamin content of the various zones was then compared on the dry-solids basis.

Enriched white bread (synthetic thiamin): A commercial enriching concentrate (containing synthetic thiamin) was thoroughly mixed to insure uniformity and then incorporated in a dough in the proportion of 1 g of concentrate to 300 g of flour. Table I gives the data obtained on the whole loaves of this series. In appearance the 10-minute loaf was definitely underbaked, whereas the 40-minute loaf was as definitely overbaked. A progressive loss of thiamin was observed as the time of baking was increased. At 10 minutes the destruction was very slight, almost within the experimental error of measurement, but at 20 minutes

TABLE I
EFFECT OF BAKING TIME ON B₁ CONTENT OF ENRICHED WHITE BREAD
(SYNTHETIC THIAMIN)

Length of baking	Weight of loaf	Total FSS	Sulfite blank	Thiamin	Thiamin, average of two ¹	Loss of thiamin
min	g	μg/loaf	μg/loaf	μg/loaf	μg/loaf	%
0 (dough)	—	2219	173	2046	—	—
10	504	2140	186	1954	1975	3
20	485.5	2040	186	1854	1780	13
30	464.5	1890	275	1615	1620	21
40	448	1710	390	1320	1370	33

¹ Average of whole loaf and dissected loaf (see Table II).

the destruction was 13%. At 30 minutes the thiamin loss was 21% and since this loaf was a well-baked one, 21% should represent the maximum loss to be expected in normal bakery practice.

The magnitude of the sulfite blank was increased as thiamin was inactivated by increased baking time, which indicates that a portion of the thiamin is split as it is inactivated. The increase in sulfite blank was not equivalent to the thiamin destroyed but equaled only 29% in the 40-minute bake. An increase in sulfite blank, or an unusually high sulfite blank, indicates that thiamin destruction has occurred. If one determines only FSS (fermentation stimulating substances) without sulfite correction, erroneous conclusions may be reached. If the destruction of thiamin in the 40-minute bake (Table I) is calculated from the FSS figures, one obtains only 23% destruction compared with 33% based on thiamin content.

The analyses of crust and crumb fractions (Table II) indicate that the major part of thiamin destruction occurs in the crust. The thiamin content of the crust is reduced by 50% as the time of baking is extended

TABLE II

EFFECT OF BAKING TIME ON THIAMIN CONTENT OF CRUST AND CRUMB OF ENRICHED WHITE BREAD (SYNTHETIC THIAMIN)

Time of baking	Weight of loaf	Weight of fraction, dry-solids basis ¹	FSS, dry-solids basis	Sulfite blank, dry-solids basis	Thiamin, dry-solids basis	Thiamin per loaf (calc)
<i>min</i>	<i>g</i>	<i>g</i>	<i>μg/g</i>	<i>μg/g</i>	<i>μg/g</i>	<i>μg</i>
10	498	O 106.7	7.18	0.66	6.52	2002
		M 97.5	7.43	0.74	6.69	
		I 99.9	7.17	0.62	6.55	
20	481	O 115.7	6.16	1.02	5.14	1705
		M 100.5	6.58	0.76	5.82	
		I 87.5	6.69	0.70	5.99	
30	465	O 107	5.61	1.21	4.40	1626
		M 98.8	6.89	0.95	5.94	
		I 96.98	6.68	0.83	5.85	
40	449	O 110	4.66	1.45	3.21	1422
		M 103.7	6.60	1.00	5.68	
		I 88.2	6.42	0.99	5.43	

¹ O signifies the third of loaf including the crust. M signifies the third of loaf between crust and innermost crumb. I signifies the third of loaf including the innermost crumb.

from 10 to 40 minutes, whereas in the same time the crumb has lost only 17% of its thiamin. The progressive increase in sulfite blank is also observed in this series, and as might have been expected the increase is greater in the crust where the greater thiamin destruction has occurred.

Whole-wheat bread: A series of loaves was baked with whole-wheat flour. The results (Tables III and IV) indicate that thiamin destruction occurs to the same general extent as in enriched white bread. It appears that the difference in origin of the thiamin has no noticeable effect upon its stability in the baking of bread.

Enriched white bread (high B₁ yeast): High vitamin B₁ white bread with yeast as the source of the vitamin was baked by substituting high B₁ yeast in the formula. The results of this series (Tables V and VI) are substantially the same as obtained with whole wheat bread and with white bread enriched with synthetic thiamin.

Bakery scale experiment: The laboratory-baked loaves were baked for fixed lengths of time—10, 20, 30, and 40 minutes—regardless of the fact that the loaf was underbaked at 10 and overbaked at 40 minutes. In order to compare the various types of bread under normal bakery conditions, 50-pound doughs were baked in a full-size electric bakery oven, being handled in the usual manner to produce normal loaves of bread. The sponge dough process was employed. Samples for analysis were taken from the dough stage, at which time the dough was

TABLE III

EFFECT OF BAKING TIME ON THIAMIN CONTENT OF WHOLE-WHEAT BREAD

Time of baking	Weight of loaf	Total FSS	Sulfite blank	Thiamin	Thiamin, average of two ¹	Loss of thiamin
<i>min</i>	<i>g</i>	<i>μg/loaf</i>	<i>μg/loaf</i>	<i>μg/loaf</i>	<i>μg/loaf</i>	<i>%</i>
0 (dough)		1921	173	1748	—	—
10	517	1940	207	1733	1708	2
20	499	1872	364	1508	1446	17
30	480	1760	367	1393	1352	23
40	468.5	1632	444	1188	1167	33

¹ Average of whole loaf and dissected loaf (see Table IV).

TABLE IV

EFFECT OF BAKING TIME ON THIAMIN CONTENT OF CRUST AND CRUMB OF WHOLE-WHEAT BREAD

Time of baking	Weight of loaf	Weight of fraction, dry-solids basis ¹	FSS, dry-solids basis	Sulfite blank, dry-solids basis	Thiamin, dry-solids basis	Thiamin per loaf (calc)
<i>min</i>	<i>g</i>	<i>g</i>	<i>μg/g</i>	<i>μg/g</i>	<i>μg/g</i>	<i>μg</i>
10	512.5	O 110	6.07	0.98	5.09	1682
		M 99	6.52	0.61	5.91	
		I 97	6.27	0.74	5.53	
20	496.5	O 117	5.38	1.17	4.21	1384
		M 90	5.61	1.09	4.52	
		I 99	5.89	1.03	4.86	
30	480	O 118	4.65	1.27	3.38	1312
		M 92	6.04	1.28	4.76	
		I 94	6.03	1.03	5.00	
40	466.5	O 125	4.28	1.58	2.7	1146
		M 93	5.84	1.39	4.45	
		I 87	5.92	1.39	4.53	

¹ O signifies the third of loaf including the crust. M signifies the third of loaf between crust and innermost crumb. I signifies the third of loaf including the innermost crumb.

TABLE V

EFFECT OF BAKING TIME ON THIAMIN CONTENT OF ENRICHED WHITE BREAD (HIGH B₁ YEAST)

Time of baking	Weight of loaf	Total FSS	Sulfite blank	Thiamin	Thiamin, average of two ¹	Loss of thiamin
<i>min</i>	<i>g</i>	<i>μg/loaf</i>	<i>μg/loaf</i>	<i>μg/loaf</i>	<i>μg/loaf</i>	<i>%</i>
0 (dough)		2375	183	2192	—	—
10	498.5	2352	217	2135	2052	6
20	482	2248	320	1928	1877	14
30	461.5	2064	358	1706	1683	23
40	445.5	1968	396	1572	1503	32

¹ Average of whole loaf and dissected loaf (Table VI).

TABLE VI
EFFECT OF BAKING TIME ON THIAMIN CONTENT OF CRUST AND CRUMB OF ENRICHED
WHITE BREAD (HIGH B₁ YEAST)

Time of baking	Weight of loaf	Weight of fraction, dry-solids basis ¹	FSS, dry- solids basis	Sulfite blank, dry- solids basis	Thiamin, dry-solids basis	Thiamin per loaf (calc)
<i>mm</i>	<i>g</i>	<i>g</i>	<i>μg/g</i>	<i>μg/g</i>	<i>μg/g</i>	<i>μg/loaf</i>
10	495	O 95.6	7.33	1.21	6.12	1969
		M 99.7	7.84	1.08	6.76	
		I 108	7.55	0.97	6.58	
20	478.5	O 99.3	6.34	1.39	4.95	1826
		M 100	7.49	1.20	6.29	
		I 103.3	7.78	0.96	6.82	
30	462.5	O 104.4	5.49	1.40	4.09	1659
		M 102.1	7.27	1.16	6.11	
		I 967	7.29	1.02	6.27	
40	450.5	O 109.1	4.96	1.66	3.30	1434
		M 100.2	6.89	1.42	5.47	
		I 92.9	7.15	1.35	5.80	

¹ O signifies the third of loaf including the crust. M signifies the third of loaf between crust and innermost crumb. I signifies the third of loaf including the innermost crumb.

weighed. The yield of bread was also determined (one hour out of the oven) and the relation of dough to bread could then be calculated. The results are given in Table VII, from which it appears that destruc-

TABLE VII
STABILITY OF THIAMIN IN BREAD BAKED ON A COMMERCIAL SCALE

	Dough thiamin ¹	Bread thiamin	Thiamin loss
	<i>μg/loaf</i>	<i>μg/loaf</i>	<i>%</i>
White bread (high B ₁ yeast)	1882	1469	22
White bread (synthetic thiamin)	2140	1687	21
Whole wheat bread	1887	1334	26

¹ Calculated on the basis of observed bread yield per unit weight of dough.

tion in these "normally" baked loaves approximated that observed in the laboratory bakes at 30 minutes.

Summary

The stability of thiamin in the baking of bread was not affected by the source of thiamin. Synthetic thiamin, high vitamin bakers' yeast, and whole wheat were the sources used.

The extent of thiamin destruction was markedly influenced by the time of baking. Loaves of "normal" bake showed a thiamin loss of approximately 20%.

The major portion of thiamin destruction occurred in the crust although destruction in the innermost crumb was not insignificant. Results with large-scale bakery experiments agreed with the laboratory results.

Acknowledgment

The laboratory bakes were made by Mr. Stanley A. McHugh, and the large-scale bakes by Mr. Harry Ekstedt. We wish to express our thanks to both of these men for their willing cooperation.

Literature Cited

- Dawson, E. R., and Martin, G. W.
1941 Vitamin B₁ estimation in yeast and bread and stability during bread-making. *J. Soc. Chem. Ind.* **60**: 241-245.
- Food and Drug Administration
1941 Bread Hearings. Washington, D. C.
- Hoffman, C., Schweitzer, T. R., and Dalby, G.
1940 The loss of thiamin in bread on baking and toasting. *Cereal Chem.* **17**: 737-739.
- Schultz, A. S., Atkin, L., Frey, C. N., and Williams, R. R.
1941 Application of the sulfite cleavage of thiamin to the yeast fermentation method. *J. Am. Chem. Soc.* **63**: 632.
- Schultz, A. S., Atkin, L., and Frey, C. N.
1942 The determination of vitamin B₁ by the yeast fermentation method. Improvements related to the use of sulfite cleavage and a new fermentometer. *Ind. Eng. Chem. (Anal. Ed.)* **14**: 35-39.

BOOK REVIEW

Modern Bread from the Viewpoint of Nutrition. By Henry C. Sherman and Constance S. Pearson. 104 pages. Macmillan Company, New York. Price \$1.75.

According to the jacket, "If it is your job to plan diets or to advise others in the management of a food budget with a view to keeping down cost without sacrificing the essentials of adequate nutrition, you will want this book." From the Columbia University Nutrition Laboratories comes this latest discussion of the place of bread in modern nutrition. The authors describe the importance of bread as a source of energy, proteins, minerals, and vitamins and discuss means by which these values may be enhanced. A discussion of the role to be played by enriched flour (written in August, 1941) and the value of the enriching compounds and elements is included. The improvement of nutritive values observable when dried milk is used in bread making is stressed. The authors point out that calcium might well be considered a valuable adjunct in addition to thiamin, nicotinic acid, and iron. They conclude that when flour is properly supplemented with vitamins and minerals by the addition of synthetic compounds, or when it contains vitamins and minerals by virtue of residual bran and germ, bread made therefrom with dried milk may logically be used to furnish as much as 40% of the total calories in the diet. There are 84 literature references.

H. S. OLCOTT, Western Regional Research Laboratory,
Bureau of Agricultural Chemistry and Engineering,
U. S. Department of Agriculture,
Albany, California

CEREAL CHEMISTRY

VOL. XIX

SEPTEMBER, 1942

No

THE THIAMIN, RIBOFLAVIN, NICOTINIC ACID, AND PANTOTHENIC ACID CONTENTS OF WILD RICE (*ZIZANIA AQUATICA*)¹

J. WESLEY NELSON and LEROY S. PALMER

Division of Agricultural Biochemistry, University of Minnesota,
St. Paul, Minnesota

(Received for publication January 29, 1942)

Kennedy (1924) found that wild rice is an adequate source of B vitamins for normal growth of rats at 86% level in the diet but no analyses have been reported of the individual components of the vitamin B complex in this cereal since the discoveries regarding their chemical identity and the perfecting of simple, accurate methods for their quantitative determination. The present study was undertaken at the request of Mr. James W. Kauffman of the U. S. Department of the Interior, Office of Indian Affairs Field Service, Minneapolis, Minnesota, because of the importance of wild rice as a native crop harvested by the Indians of Minnesota. The samples analyzed were obtained by Mr. Kauffman and are representative of the parched product processed by the Indians and marketed by them.

Thiamin was determined by the thiochrome method of Hennessy (1941), riboflavin by the microbiological method of Snell and Strong (1939) as modified by Andrews, Boyd, and Terry (1941), nicotinic acid by the microbiological method of Snell and Wright (1941), and pantothenic acid by the microbiological method of Strong, Feeney, and Towner (1941).

The results shown in Table I indicate that parched wild rice may be regarded as a good source of thiamin, riboflavin, and nicotinic acid for human nutrition. It appears to be a surprisingly good source of thiamin since 100 g contain about 40% of the recommended allowance for a person requiring 2500 kilo-calories.² The daily allowances are favorably with wheat, corn, and rye in thiamin content. Wild rice is considerably richer in riboflavin than wheat, corn, oats, and is comparable with rice.

¹ Paper No. 1961, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul, Minnesota. Accepted for publication, May 26, 1942.
² Committee on Food and Nutrition, National Research Council, Washington, D. C., May 26, 1942.
³ Recommendations of the National Conference, Washington, D. C., May 26, 1942.

TABLE I
B-COMPLEX VITAMINS IN WILD RICE

Sample	Crop year	Vitamin content per 100 g			
		Thiamin	Riboflavin	Nicotinic acid	Pantothenic acid
		mg	mg	mg	mg
No. 1	1939	0.507	0.542	6.69	0.98
No. 2	1939	0.579	0.700	5.48	1.31
Screenings	1939	0.371	1.058	6.34	1.41
Nett Lake	1940	0.293	0.417	6.58	0.65
Nett Lake (light)	1940	0.371	0.708	5.58	1.67
Mitchell Dam	1940	0.600	0.800	6.44	0.42
Average (exclusive of screenings)	—	0.470	0.633	6.13	1.01

Literature Cited

- Andrews, J. S., Boyd, H. M., and Terry, D. E.
1941 Application of the microbiological method to the riboflavin analysis of cereals. Abstracts Division of Biological Chemistry, Am. Chem. Soc. 102nd Meeting, Atlantic City, Sept. 8-12, p. 36.
- Hennessy, D. J.
1941 Chemical methods for determination of vitamin B₁. Ind. Eng. Chem. (Anal. Ed.) 13: 216-218.
- Kennedy, Cornelia
1924 The nutritive properties of wild rice (*Zizania Aquatica*). J. Agr. Res. 27: 219-224.
- Snell, E. E., and Strong, F. M.
1939 A microbiological assay for riboflavin. Ind. Eng. Chem. (Anal. Ed.) 11: 346-350.
- Snell, E. E., and Wright, L. D.
1941 A microbiological method for the determination of nicotinic acid. J. Biol. Chem. 139: 675-686.
- Strong, F. M., Feeney, R. E., and Earle, Ann
1941 Microbiological assay for pantothenic acid. Ind. Eng. Chem. (Anal. Ed.) 13: 566-570.

A CALCULATED BLANK FOR THE ESTIMATION OF THIAMIN BY THE FERMENTATION METHOD

I. ENRICHED BREAD

GEORGE J. LAEMMLE and O. W. BARLOW

Research Laboratories, Winthrop Chemical Company, Inc., Rensselaer, New York
(Received for publication May 23, 1942)

assay of thiamin by the yeast fermentation method proposed by H. H. Altz, Atkin, and Frey (1937), the apparatus consisted of twelve flasks shaken in a constant-temperature bath and twelve gasometers. Since the amount of thiamin in the flasks, and since the amount of

sample requires the simultaneous determination of the sulfite blank of the sample in the same apparatus, as shown by Schultz, Atkin, Frey, and Williams (1941), no more than five samples can be assayed at one time. We have found it possible to complete two separate fermentation runs in an eight-hour day, permitting the assay of as many as ten samples for thiamin content by this procedure as a matter of daily routine. In this respect the yeast fermentation method suffers in comparison to thiamin assay by the thiochrome procedure described by Hennessy and Cerecedo (1939) and modified by Hennessy (1941) and by Connor and Straub (1941). Sixteen to twenty samples a day may be assayed by this method.

The omission or elimination of the sulfite blank in the yeast fermentation method would not only double the capacity of the apparatus employed, but would also result in a considerable decrease in the time required for a fermentation run, since much of the assay time represents the preparation of the sulfite blank. This blank, however, cannot be disregarded as it often comprises a very considerable part of the total fermentation-stimulating substances. Nor is the blank constant for any class of material assayed but varies with different samples and even at times with duplicate determinations, although the *net* thiamin in each of the duplicates is in close agreement. We have observed this variability of the sulfite blank in all kinds of material assayed by this method.

In laboratories where the same material, prepared according to an unchanging process and formula, is routinely assayed, it may be feasible to omit the sulfite blank and to substitute a standard sample of the product which has been carefully assayed by one or more methods. All the samples are then corrected by the factor necessary to adjust the apparent thiamin content of the standard to the known true value. In laboratories where many different materials are assayed, or where the same general material is received from different sources, such a procedure cannot be adopted.

We have for some time observed a limited relationship between the sulfite blank and the total apparent thiamin (or total fermentation-stimulating substances). When two different samples of the same general material are assayed by the fermentation procedure, the sample having the greater apparent thiamin content will usually have the greater sulfite blank. This is especially noticeable with a material like enriched bread, wherein the variation in thiamin content among individual samples is greater than that encountered in pharmaceuticals produced under controlled conditions, or in unfortified natural materials. When, therefore, it seemed possible that this ratio between the sulfite blank and the total apparent thiamin, if compiled for a

large number of samples of the same general material, might prove to be constant within the experimental error of the method itself, enriched bread appeared to be the material best suited to investigation.

Experimental

The ratio between the sulfite blank and the total apparent thiamin, both figures being expressed in micrograms of thiamin per gram of air-dry sample, was determined for 100 unselected samples of enriched bread. In order to obtain the most trustworthy picture of variations in this ratio, the series was made up of breads taken in the order received for assay without omission of any sample. The frequency distribution of the ratio blank: fermentation-stimulating substance (f.s.s.) for this series of breads is shown in Figure 1.

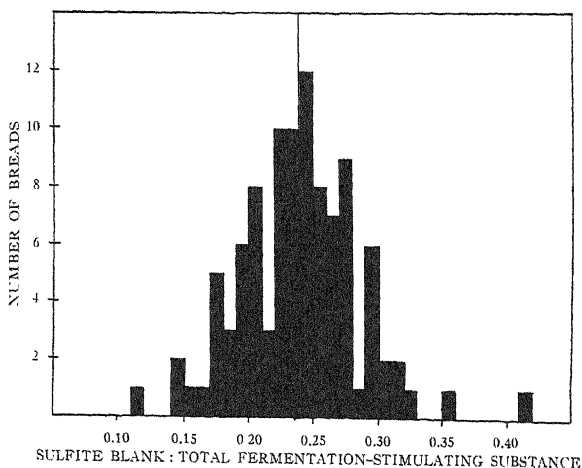


Fig. 1. Frequency distribution of the ratio of sulfite blank to total f.s.s.
Average ratio, 0.235. $\sigma = 0.047$.

The frequency distribution of this ratio did not afford evidence of any constancy sufficient to permit the use of an empirical correction factor, determined in such fashion, in place of the sulfite blank. The standard deviation of the series was almost 20% of the mean value, and the individual ratios ranged from a low of 0.109 to a high of 0.411.

There were, however, two encouraging aspects of the frequency distribution. First, the distribution followed fairly closely that predicted from the normal curve of error, as shown in Table I. This agreement is evidence that the variation in the ratio of the blank to the total fermentation stimulation is due to chance unavoidable errors

TABLE I
VALUES FOR NORMAL ERROR CURVE

Deviation from the mean in terms of σ	Number of breads in range of error predicted from normal curve	Found
$\pm 0.6745 \sigma$	50	54
$\pm \sigma$	68	72
$\pm 1.4 \sigma$	84	87
$\pm 1.7 \sigma$	91	93
$\pm 2.2 \sigma$	97	97

in the method and technique, rather than to real differences in the ratio among individuals in the series. Second, and more important, is the fact that the blank averages only 24% of the total increase in the fermentation rate. Therefore a comparison of the thiamin content of the members of the series determined with the factor 0.238 f.s.s. as the blank, with the thiamin content as determined in the usual manner, will result in a much smaller standard deviation than that obtained when the same series was used to determine the average ratio, 0.238. This fact is demonstrated graphically in Figure 2.

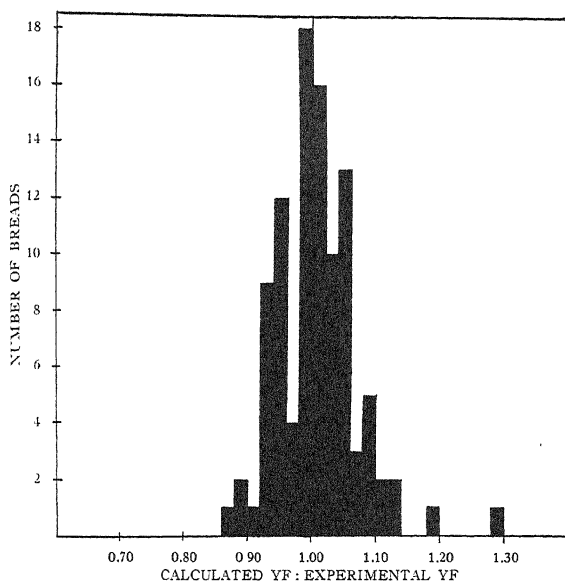


Fig 2. Frequency distribution of the ratio of thiamin contents determined from the calculated blank (0.238 f.s.s.) and from the actual sulfite blanks. Average ratio, 1.002. $\sigma = 0.063$.

In this comparison, the calculated and experimental methods agreed within $\pm 5\%$ for 70% of the series, and they agreed within $\pm 10\%$ for 92% of the series.

It is important to realize that in this effort to establish a sound basis for the use of a calculated blank, by statistical methods, we are not studying the distribution of experimental observations around a known true value. Rather, we have a picture of the variance of a series of calculated results about an experimentally determined mean which is subject to all the uncertainties inherent in the method and in the technique of the analyst. Therefore it is possible that the calculated blank, based on a large number of determinations, is more reliable than the individual experimental blank which it supplants. This possibility was studied in the following way.

Ten enriched breads which were not members of the series previously studied were assayed by the yeast fermentation method, employing the usual procedure for determination of the sulfite blank. The same group of breads was then assayed by the same procedure, omitting the sulfite blank. The blank in this case was calculated as 0.238 f.s.s. Finally the ten breads were assayed by the thiochrome method. The results obtained by the three procedures are tabulated in Table II.

TABLE II
THIAMIN ASSAY BY THREE METHODS

Sample No	Thiamin air-dry basis			Thiochrome Sulf YF	Deviation	Thiochrome Calc YF	Deviation	Calc YF Sulf YF	Deviation
	Sulf YF	Thiochrome	Calc YF						
	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	%	%	%	%	%	%
1608	3.16	3.03	2.90	96	-4	104	+4	92	-8
1611	3.18	2.96	3.18	93	-7	93	-7	100	0
2098	2.98	2.88	2.85	97	-3	101	+1	96	-4
2099	3.23	3.38	3.29	104	+4	103	+3	102	+2
2188	4.69	4.49	4.59	96	-4	98	-2	98	-2
2193	3.55	3.69	3.55	104	+4	104	+4	100	0
2253	2.71	2.60	2.76	96	-4	94	-6	102	+2
2254	2.88	2.60	2.90	90	-10	90	-10	99	-1
2289	3.69	3.46	3.49	94	-6	99	-1	94	-6
2335	3.19	3.15	3.18	99	-1	99	-1	100	0
Average	3.33	3.22	3.27	96.9	± 4.7	98.5	± 3.9	98.3	± 2.5

Sulf YF = yeast fermentation with sulfite blank. Calc YF = yeast fermentation with calculated blank.

The closest agreement found was that between the fermentation assay with the calculated blank and the usual fermentation procedure. The former procedure yielded results which were in somewhat closer agreement with those obtained by the thiochrome method than were the latter, but the deviation in all three assays is sufficiently within

the experimental error of the methods to preclude the assumption of a significant difference in the reliability of any of the procedures. However, the calculated blank stands up well in comparison with another standard assay method.

In a preliminary study of a limited number of non-enriched breads we have found the ratio between the sulfite blank and the total fermentation-stimulating substances to be considerably higher than that found for the enriched breads studied. Such a finding suggested that this ratio would decrease as the enrichment level of the bread was increased. However, a breakdown of the series from which the average ratio was calculated, according to thiamin content, demonstrated no essential variation in the ratio over a considerable range of enrichment (Table III).

TABLE III
EFFECT OF ENRICHMENT LEVEL

Thiamin	No. breads	Calc YF Exptl YF	Average deviation
$\mu\text{g/g}$		%	+
1-2	2	104.5	± 0.5
2-3	39	102.1	± 5.6
3-4	54	98.4	± 3.9
4-5	1	99	- 1
8-9	2	103	± 1
12-13	2	92.5	± 0.5

A comparison of the ratio calc YF : exptl YF obtained with those members of the series known to have been fortified with enriched yeast and with those breads known to have been enriched with crystalline thiamin, revealed no significant difference in either the mean ratio or average deviation of the mean (Table IV).

TABLE IV
EFFECT OF METHOD OF ENRICHMENT

Number of breads	Method of enrichment	Calc YF Exptl YF	Average deviation
13	Crystalline thiamin	0.976	± 0.060
15	Enriched yeast	1.032	± 0.055

It is unfortunate that for the majority of the series the method of enrichment was unknown or not known with certitude.

Conclusions

It is to be expected that the empirical ratio determined from the series of enriched breads will change in value with the inclusion of a

greater number of breads in the series, or when calculated from a new series of experimental results. However, the agreement in results obtained with the experimental and the calculated sulfite blanks, illustrated in Table IV, makes improbable any change in the factor considerable enough to invalidate its use. It is also possible that this ratio may differ widely among laboratories while remaining fairly constant for each. This variation, if demonstrated, will severely limit the practical value of the calculated blank, but we believe that the agreement between laboratories in collaborative studies renders improbable such a variation. We are hopeful that a constant empirical ratio between the blank and the total apparent thiamin in the yeast fermentation method can be established, not only for enriched bread but for other assay materials, with a resulting increase in the capacity of the method and decrease in the assay time involved.

Summary

The ratio of the sulfite blank to the total fermentation-stimulating substances, in a series of 100 enriched bread samples assayed for thiamin by the yeast fermentation procedure, was found to be 0.238. Substitution of this empirical factor for the individual sulfite blanks doubles the capacity of the method and brings about a considerable reduction in assay time. The results obtained with the calculated blank are in close agreement with those obtained in the usual way.

Literature Cited

- Connor, R. T., and Straub, G. J.
1941 Determination of thiamin by the thiochrome reaction. *Ind. Eng. Chem.* **13**: 380-384.
- Hennessy, D. J.
1941 Chemical methods for the determination of vitamin B₁. *Ind. Eng. Chem. (Anal. Ed.)* **13**: 216-218.
- Hennessy, D. J., and Cerecedo, L. R.
1939 The determination of free and phosphorylated thiamin by a modified thiochrome assay. *J. Am. Chem. Soc.* **61**: 179-183.
- Schultz, A. S., Atkin, L., and Frey, C. N.
1937 A fermentation test for vitamin B₁. *J. Am. Chem. Soc.* **59**: 948-949.
1937 A fermentation test for vitamin B₁-II. *J. Am. Chem. Soc.* **59**: 2457-2460.
- Schultz, A. S., Atkin, L., Frey, C. N., and Williams, R. R.
1941 Application of the sulfite cleavage of thiamin to the yeast fermentation method. *J. Am. Chem. Soc.* **63**: 632.

THE DISTRIBUTION OF THIAMIN IN THE WHEAT PLANT AT SUCCESSIVE STAGES OF KERNEL DEVELOPMENT ¹

W. F. GEDDES ² and M. N. LEVINE ³

(Read at the Annual Meeting, May 1941)⁴

In 1940, a three-year WPA project was initiated cooperatively by the Division of Plant Pathology and Botany and the Division of Agricultural Biochemistry of the University of Minnesota for the purpose of studying the effects of leaf rust (*Puccinia rubigo-vera tritici*) and stem rust (*Puccinia graminis tritici*) on the agronomic properties of spring wheat, the translocation of plant constituents into the developing kernel, and the industrial quality and chemical composition of the resulting grain. This was an extension of a study begun in 1935. The problem has been attacked from two directions, by the so-called inhibitive and the preventive methods of rust control. In the inhibitive series, the rust epidemics were produced in the early stages of plant growth and arrested at given periods by sulfur dusting; in the preventive series, the incidence of rust was excluded by sulfur dusting until certain stages in plant development were reached, after which artificial inoculations were made. Thatcher was used for the leaf-rust and Ceres for the stem-rust experiments.

Current interest in the thiamin content of wheat and the scarcity of information regarding thiamin levels at different stages of maturity warrant the presentation of data on samples from the plots in each series where the incidence of rust was controlled by sulfur dusting throughout the growth and ripening of the plants.

Two dates of planting were employed for the leaf rust studies: April 25 and May 21; and one planting date only, May 21, was used for the study of the effect of stem rust. Sampling of the plots for the chemical studies was begun seven days after blossoming and continued at successive three- and four-day intervals until maturity.

The entire aerial portions of individual tillers were collected, their green weights determined, and the heads clipped off and weighed. The two plant fractions, one comprising the stems and adhering leaves, and the other the heads, were dried at approximately 60°C and their dry weights recorded. The heads were then threshed quantitatively,

¹ Joint contribution from the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Divisions of Plant Pathology and Botany and Agricultural Biochemistry, University of Minnesota. Paper No. 2,001, Scientific Journal series, Minnesota Agricultural Experiment Station. Grateful acknowledgment is hereby made to the personnel of the Work Projects Administration, Official Project No. 165-1-71-124, sponsored by the University of Minnesota, for assistance in carrying out the experimental work.

² Professor of Agricultural Biochemistry, Department of Agriculture, University of Minnesota.

³ Pathologist, U. S. Department of Agriculture, detailed for cooperative work in the Division of Plant Pathology and Botany at the University of Minnesota.

⁴ Additional data have been accumulated subsequent to the presentation at the annual meeting and are incorporated in this paper.

and the air-dry weights of the kernels and of the nonkernel portion (glumes and rachis) were determined. In this manner three plant fractions, namely (1) kernels, (2) glumes and rachis, and (3) stems and leaves, were provided for the chemical studies. Since the weights of each fraction and the number of tillers represented were known, it was possible not only to express the results of all analytical determinations on a percentage basis but also to calculate the quantities and percentage distribution of each constituent in the three plant fractions as well as the percentage and total amount in the entire tiller at successive stages of kernel development.

Experimental Results

After each plant fraction was ground in a Wiley laboratory mill to pass the 0.5-mm sieve, thiamin was determined by the thiochrome method, as outlined in *Cereal Laboratory Methods* (4th edition, 1941). The mean results are recorded in Table I, together with data for the moisture content of the entire tillers and the weight per thousand kernels, which serve as indices of the stage of maturity.

The results recorded for the early-sown Thatcher are the means of duplicate determinations on two sets of samples, comprising the controls for both the preventive and inhibitive series but representing identical treatments, while those given for the late-sown Thatcher and Ceres are the means for one set of samples, comprising in each case the controls for the preventive series alone. From the practical standpoint, the data for early-sown Thatcher are of the greatest interest and are diagrammatically represented in Figure 1.

In each test similar trends occurred in the thiamin content of the three plant fractions with progressing maturity. The most important phenomenon elucidated by these studies is the fact that most of the thiamin ultimately found in the plant was present soon after blossoming and that thereafter the thiamin was translocated from the glume and stem fractions into the developing kernels.

On a unit weight basis, expressed as micrograms per gram of dry matter, the thiamin content of the kernels decreased rather consistently during the first few weeks after blossoming and then remained relatively constant, with an apparent tendency to rise slightly at full maturity. The initial relatively rapid decrease may be explained by the fact that the endosperm is low in thiamin and hence, as kernel filling proceeds, the concentration in the entire kernel tends to decrease. Sherwood, Nordgren, and Andrews⁵ have shown that the thiamin of the mature wheat kernel is largely concentrated in the germ

⁵ R. C. Sherwood, R. Nordgren, and J. S. Andrews: Thiamin in the products of wheat milling and bread, *Cereal Chem.* 18: 811-819, 1941.

TABLE I
MOISTURE CONTENT, WEIGHT PER THOUSAND KERNELS, THIAMIN CONTENT, AND THIAMIN DISTRIBUTION
IN THATCHER WHEAT AT SUCCESSIVE STAGES OF DEVELOPMENT

Date of sampling	Days after blossoming	Weight per 1,000 kernels (dm basis) ¹	Thiamin content in micrograms per gram (dm basis)				Total thiamin in micrograms				Distribution of thiamin		
			Kernels	Glumes and rachis	Stems and leaves	Entire tiller	Kernels	Glumes and rachis	Stems and leaves	Entire tiller	Kernels	Glumes and rachis	Stems and leaves
		g	μg/g	μg/g	μg/g	μg/g	μg	μg	μg	μg	c ₁	c ₂	c ₃
EARLY-SOWN THATCHER (AVERAGE LEAF RUST = 11%) ³													
July 8	7	5.4	69.0	7.7	2.8	2.1	2.6	1.2	3.0	4.9	24.7	15.0	60.3
12	11	9.7	67.2	7.4	2.2	2.0	2.9	2.4	3.0	6.0	39.9	10.3	49.8
15	14	12.7	65.2	6.0	1.9	1.8	2.7	2.7	2.8	6.0	44.8	9.0	46.2
19	18	17.0	62.0	5.6	1.5	1.6	2.6	3.3	2.4	6.1	53.9	7.2	38.9
22	21	23.4	55.8	6.1	1.0	1.5	3.0	4.8	2.0	7.1	67.3	3.9	28.8
25	24	26.0	52.0	6.2	0.8	1.4	3.0	5.1	1.8	7.0	71.9	3.1	25.0
29	28	26.6	47.8	6.3	0.6	1.3	2.9	4.6	1.5	6.3	74.2	2.5	23.3
Aug. 1	31	26.7	43.2	6.5	0.5	1.2	2.9	4.5	1.2	5.8	77.4	2.0	20.6
LATE-SOWN THATCHER (AVERAGE LEAF RUST = 16%) ³													
July 22	7	3.4	68.6	7.2	3.3	3.2	3.5	0.9	4.5	6.5	13.6	17.3	69.1
25	10	6.5	68.3	7.6	2.8	3.1	3.6	1.9	4.2	7.0	26.5	13.7	59.6
29	14	12.7	63.9	6.6	1.7	2.7	3.5	3.4	3.4	7.3	45.6	7.5	46.9
Aug. 1	17	15.3	62.4	5.9	1.5	2.4	3.3	3.6	2.9	6.9	51.9	6.6	41.5
5	21	19.2	56.6	5.7	1.4	2.1	3.4	4.3	2.2	7.0	63.0	5.6	31.4
8	24	20.7	53.8	5.2	1.1	1.8	3.0	3.9	1.8	6.0	65.3	5.0	29.7
12	28	23.0	46.8	5.2	0.6	1.2	2.8	4.2	1.1	5.5	76.2	3.1	20.7
LATE-SOWN CERES (AVERAGE STEM RUST = 5%) ³													
July 22	7	5.6	65.0	8.2	4.0	2.5	3.3	1.4	3.8	7.2	19.2	27.3	53.5
25	10	9.9	63.6	7.8	2.8	2.2	3.2	3.1	3.4	7.8	39.3	17.2	43.5
29	14	14.9	60.1	6.5	2.2	1.7	2.9	3.6	2.1	6.6	53.6	14.2	32.1
Aug. 1	17	18.0	58.2	6.3	1.7	1.5	2.9	4.1	1.8	6.6	62.0	10.6	27.4
5	21	19.5	48.7	6.6	1.1	1.0	2.9	5.1	1.2	6.7	75.8	6.7	17.5
8	24	20.8	37.7	6.4	1.0	1.0	2.8	4.8	1.1	6.3	76.6	6.2	17.2

¹ Mean of duplicate determinations of the weight of 500 air-dry kernels. Moisture determined by drying a ground air-dry sample to constant weight in a vacuum oven at 100°C.

² Computed from the percentage of moisture lost on air-drying and the vacuum-oven moisture values for the three plant fractions.

³ The severity of rust infection was determined with the aid of the "Scale for Estimating Rust" devised by the U.S.D.A. The average infections shown for the early sown grain were obtained from three semi-weekly readings; those for late-sown grain were based on two such readings.

and in the tissues just beneath the outer bran, that is, in tissues which are laid down early in kernel development.

The progressive decrease in the thiamin concentration in the glume and stem fractions, especially the former, was very striking; thus in mature plants the thiamin concentration of the glume fraction was only 18% to 25% of what it was one week after blossoming, while in the stem fraction the reductions amounted to from 38% to 57% of the earlier figures. In considering the data for the thiamin concentration of the entire tiller, it must be emphasized that errors in the dry-matter weights and thiamin values for the three plant fractions are accumu-

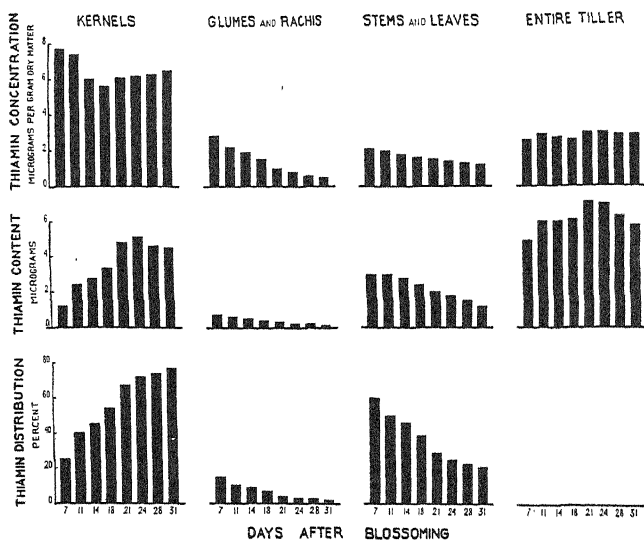


Fig. 1. Diagram showing the concentration, content and distribution of thiamin in different fractions of the wheat plant at successive stages of kernel development.

lated in the computation of the values for the tiller as a whole. In general, however, it would appear that the thiamin concentration of the entire tiller changes only slightly, with progressing maturity; the early-sown Thatcher series showed no definite trend, but with late-sown Thatcher and Ceres there is some indication of a slight decrease as the plant matures.

The thiamin values per unit of dry matter by themselves do not give a complete picture, since the relative weights of the three plant fractions change as the plant matures, the kernels increasing and the glume and stem fractions decreasing in weight. When dry-matter

weights per individual tiller are taken into consideration, that is, when the comparisons are made on the basis of total thiamin, the thiamin content of the kernels increased progressively until the kernel was almost fully matured, after which a slight decrease is noted. This decrease may, however, be only apparent due to loss of kernels through shattering and the depredations of birds. In striking contrast to the kernels, the total thiamin content of the glumes and stems, notably the latter, markedly decreased throughout the entire maturation period.

Considering the data for all three series, the thiamin content of the entire tiller showed no definite trend, fluctuating somewhat in the various sampling periods. As already noted, errors in the thiamin concentrations and dry-matter weights of the three fractions are accumulated in computing these values. The indicated decrease in the thiamin content of the tiller for the last few sampling periods was due, in part at least, to some loss of leaves and also of kernels from the semi-ripe plants. However, the data do indicate that the bulk of the thiamin found in the mature plant is present about one week after blossoming, and as maturity progresses this is translocated from the glume and stem fractions into the kernels.

The translocation of thiamin from the glumes and stems is conspicuously marked by the changes in the distribution of the total thiamin in the three plant fractions with maturity. For example the percentage represented by the kernels in the early-sown Thatcher gradually increased from about 25% at seven days after blossoming to 77% at maturity, whereas the percentage in the glume fraction fell from 15% to 2% and in the stem fraction from about 60% to 21%. In the late-sown Thatcher and Ceres the proportion of thiamin in the kernels was initially somewhat lower, apparently as a result of relatively greater immaturity, but the final values for the kernels were very similar in all series. It is of interest to note that with Ceres, a bearded wheat, a much larger percentage of the total thiamin was present in the glumes than was the case with the beardless Thatcher wheat.

It should be emphasized that the data presented above were obtained with wheat plants in which an effort was made to eliminate the effect of leaf and stem rust on the metabolism of the plant through the control of these parasites by sulfur dusting. Analyses now in progress indicate that stem rust in particular markedly decreased the extent of translocation of the thiamin into the kernels, but a detailed consideration of the effect of leaf and stem rust must await the completion of assays of plants with varying severities of rust infection. Studies are also in progress to determine whether the application of sulfur has, in itself, any influence on the thiamin content of the wheat

plant. The data presented at this time may be regarded as an index of the course of events in the normal wheat plant and it is of particular interest that roughly 75% of the total thiamin of the aerial portions of the mature plant is found in the kernels. The complete physiological inventory of thiamin would require analyses of the roots in addition to the aerial portions but such analyses are impractical under field growing conditions.

Summary

Thiamin assays have been carried out by the thiochrome method on three plant fractions (kernels, glumes and rachis, and stems and leaves) prepared from tillers harvested during kernel filling from plots of early-sown Thatcher, late-sown Thatcher, and late-sown Ceres wheats in which leaf and stem rust were controlled by sulfur dusting. In each series, similar and very striking trends occurred in the thiamin concentration and distribution of the total thiamin in the three plant fractions with progressing maturity.

The thiamin concentration in the kernels decreased slightly during the first few weeks after blossoming, and then remained relatively constant with an apparent slight increase at full maturity. Thiamin concentration in the glume and stem fractions markedly decreased with the filling of the kernels; in the mature plant the values for the glume fractions were only 18% to 25% and those for the stem fractions only 38% to 57% of those at 7 days after blossoming. Thiamin concentration in the tillers as a whole showed little change with maturity, although a slight decrease was indicated in the late-sown Thatcher and Ceres samples.

The total thiamin present in each of the plant fractions changed markedly with maturity. Total thiamin in the kernels increased progressively until the kernel was almost fully matured, the total thiamin in the glume and stem fractions greatly decreased throughout the entire ripening period, while that of the tiller showed no definite trend after an initial slight increase between the seventh and tenth days after blossoming.

The bulk of the thiamin found in the mature plant is present within one week after blossoming; as maturation proceeds it is translocated from the glumes, rachis, stems, and leaves into the developing kernels. Thus, in the early-sown Thatcher approximately 25% of the total thiamin of the tiller was in the kernels, 15% in the glume fraction, and 60% in the stem fraction at 7 days after blossoming. In the mature tiller the kernels contained approximately 77% and the glume and stem fractions 2% and 21%, respectively.

COLLABORATIVE STUDY OF THE APPLICABILITY OF MICROBIOLOGICAL AND CHEMICAL METHODS TO THE DETERMINATION OF NIACIN (NICOTINIC ACID) IN CEREAL PRODUCTS

DANIEL MELNICK

Food Research Laboratories, Long Island City, New York

(Read at the Annual Meeting, May 1942)

On December 13, 1940, the Research Corporation, New York City, called a meeting of a large number of workers in the field of vitamin assay to discuss the applicability of chemical and microbiological methods to the determination of thiamin, nicotinic acid, and riboflavin in cereal products. The meeting was conducted with Dr. R. R. Williams as chairman; the proceedings were recorded and distributed by the Research Corporation.

The necessity for improving the recently reported methods so that they could be used as reliable control measures in the flour and bread enrichment program became apparent. Various committees were selected to study each of the analytical procedures, to plan collaborative studies, and, on the basis of the results submitted, to suggest standard procedures for use. The author was asked to serve as chairman of the Nicotinic Acid Assay Committee.¹

The study was divided into two parts. First the collaborators were free to select any of the published microbiological and chemical procedures, including whatever personal modifications they cared to adopt. On the basis of the results submitted, collaborative microbiological and chemical methods were then drawn up for use in the second phase of the study. These methods are reproduced in the Appendix to this report.

The microbiological methods depend upon the growth-stimulating properties of nicotinic acid for certain microorganisms in a medium containing all the factors essential for the growth of the microorganism except nicotinic acid. Metabolic acid production as measured titrimetrically serves as an index of growth, and the nicotinic acid content is interpolated from a standard dose-response curve as determined for each series of tests.

¹ The collaborators who participated in the nicotinic acid study, and their respective laboratories, are included in the following list. They were also members of the Committee: E. E. Snell, University of Texas, Austin, V. Cheldelin and R. R. Williams, Summit, N. J.; C. A. Elvehjem and F. M. Strong, University of Wisconsin, Madison, H. A. Waisman, University of Wisconsin, Madison, O. L. Khne, Food and Drug Administration, Washington, D. C.; W. A. Gortner and J. S. Andrews, General Mills, Minneapolis, Minn.; K. Morgareidge, National Oil Products Company, Harrison, N. J.; D. Melnick and B. L. Oser, Food Research Laboratories, Long Island City, N. Y.; H. Isbell and W. H. Sebrell, U. S. Public Health Service, Bethesda, Md.; C. N. Frey, Fleischmann Laboratories, New York, N. Y.; W. L. Sampson, Merck Institute of Therapeutic Research, Rahway, N. J.; E. B. Brown, Anheuser-Busch, Inc., St. Louis, Mo.; H. J. Cannon, Laboratory of Vitamin Technology, Chicago, Ill.; W. A. Perlzweig, Duke University, Durham, N. C.; W. J. Dann, Duke University, Durham, N. C.; R. B. Meckel, American Institute of Baking, Chicago, Ill.

The chemical methods for nicotinic acid involve strong acid hydrolysis of the test substance, or of a properly prepared extract, to convert all the nicotinic acid derivatives to free nicotinic acid, the common denominator. Preparation of the solution for the colorimetric test may be effected either by adsorption of the pigments, leaving the nicotinic acid in the filtrate, or adsorption of the nicotinic acid and its subsequent elution. The test solution is then adjusted to proper pH and three aliquots are taken. The first is simply diluted to volume to give a measure of residual color of the solution. The nicotinic acid in the second aliquot is allowed to react with cyanogen bromide and an aromatic amine to yield a yellow color which is measured in the photoelectric colorimeter. This is converted into absolute units of nicotinic acid by comparing its photometric density with the increment in photometric density of the third aliquot containing a known increment of nicotinic acid. With these procedures the collaborators assayed four samples prepared and distributed by Dr. J. S. Andrews of General Mills, Minneapolis, and Dr. C. N. Frey of the Fleischmann Laboratories, New York.

Sample No. 1 was a whole wheat flour; No. 3, a white flour; No. 8, an air-dried whole wheat bread made from Sample No. 1; and No. 9, a nicotinic-acid-enriched bread made from Sample No. 3 and then air-dried. On the basis of work reported from our laboratory (Melnick, Oser, and Siegel, 1941), indicating no loss of nicotinic acid during the fermentation of the dough and the baking of the bread, it would seem that the values for the air-dried whole wheat bread should be essentially the same as that for the whole wheat flour, the total solids content of both samples being approximately equal. The following calculations indicated the expected increment for nicotinic acid in Sample No. 9 over No. 3 to be 33 μ g per gram.

376 mg nicotinic acid added to 25 lbs flour No. 3.

25 lbs flour yield 37.6 lbs bread.

\therefore Increment = 10 mg nicotinic acid per lb bread on fresh basis.

Average total solids in bread by air-drying = 70%.

\therefore Increment = 14.3 mg nicotinic acid per lb air-dried bread.

300 g flour (in one lb fresh bread)

2% yeast added (40 mg nicotinic acid per lb)

6.00 g yeast per lb bread.

\therefore Yeast furnishes 0.55 mg nicotinic acid per lb fresh bread or 0.8 mg nicotinic acid per lb air-dried bread.

Total nicotinic acid increment in sample No. 9 over No. 3 is:

$14.3 + 0.8 = 15.1$ mg nicotinic acid per lb air-dried bread or 33 μ g nicotinic acid per g.

Thus sample No. 9 served the additional purpose of yielding information on the adequacy of the methods for determining how much nicotinic acid had been added.

Eleven collaborators submitted values using microbiological methods of assay (Snell and Wright, 1941; Dorfman, Koser, Horwith, Berkman, and Saunders, 1940). Most of the investigators were not satisfied to follow any single procedure but actually conducted extensive investigations. It is impossible in the present paper to review all the data submitted. Essentially five methods for the extraction of the nicotinic acid were used; these were mild aqueous,² exhaustive aqueous,³ acid,⁴ alkaline,⁴ and the use of preliminary enzymic digestion⁵ of the sample followed by mild aqueous extraction.

Mild aqueous extraction gave the lowest nicotinic acid values. Predigestion of the samples with enzymes gave only slightly higher figures. Maximal values, more than twice those yielded by the mild aqueous extraction procedure, were obtained on the acid or alkaline extracts. The same figures were found despite variations in concentration of the acid or alkali from 0.1 to 2.0 normality. These maximal values were approximated in the analyses of the exhaustive aqueous extracts. In almost every case, regardless of the methods of extraction, the nicotinic acid values for samples 1 and 8 were practically the same, while the nicotinic acid increment of sample No. 9 over No. 3 approximated the theoretical 33 μ g per gram.

TABLE I
MICROBIOLOGICAL NICOTINIC ACID ASSAYS ON ALKALINE EXTRACTS

Collaborator	Procedure	Samples			
		No. 1	No. 3	No. 8	No. 9
		<i>μg per gram</i>			
No. 2	Snell-Wright	65	9.9	60	41
No. 3	“	61	9.9	65	47
No. 4	“	—	—	67	45
No. 5	“	56	8.9	60	41
No. 7	“	69	8.3	63	45
No. 8	“	65	9.9	59	44
No. 11	“	—	9.4	57	39
No. 9	“	62	10.5	64	46
No. 9	Dorfman-Koser	71	12.0	61	43
Average		64	9.9	62	43

A consideration of the various reports indicated that good agreement can be expected if a standard procedure based upon alkaline extraction is used. This is supported by the values listed in Table I

² Hot aqueous extraction followed by filtration.

³ The aqueous suspensions were autoclaved at 15 lbs pressure for 30 minutes and the insoluble residues generally reextracted with boiling water one or more times.

⁴ The acid (H_2SO_4) and alkaline ($NaOH$) suspensions were autoclaved at 15 lbs pressure for 30 minutes and the neutralized suspension or extract tested.

⁵ Enzymic (takadiastase, papain, pancreatin, etc.) digestion at 40° to 50°C, the suspension heated to 100°C, and the clear filtrate tested.

which indicate, despite the use of alkali of varying normality for the extraction, good reproducibility among laboratories.

Of particular interest was the report by Collaborator No. 9, demonstrating good agreement in nicotinic acid values for cereal products by two unrelated microbiological procedures involving use of different basal media and different microorganisms.

Nine collaborators reported assays using chemical methods for the determination of nicotinic acid (Melnick, Oser, and Siegel, 1941; Dann and Handler, 1941; Melnick and Field, 1940a; Bina, Thomas, and Brown, 1941). A summary of the results of the "more reliable" chemical tests is presented in Table II. The values are in fairly good

TABLE II
NICOTINIC ACID VALUES OBTAINED BY THE "MORE RELIABLE" CHEMICAL TESTS

Collaborator	Procedure	Samples			
		No. 1	No. 3	No. 8	No. 9
No. 2	Dann-Handler	60	<i>μg per gram</i>		
No. 4	Personal modification of Melnick-Field	—	19.8	62	
No. 6	Melnick-Field	78	15.0	78	56
No. 8	Dann-Handler composite	62	13.7	63	48
	Melnick-Oser-Siegel	61	11.5	61	43
No. 13	Melnick-Field	64	14.5	64	46
	Dann-Handler composite	74	19.8	69	50
No. 14 and 15	Dann-Handler				
Average		67	15.7	66	49

agreement with each other. The figures for sample No. 8 were essentially the same as those for No. 1 and the nicotinic acid increment in No. 9 over No. 3 approximated the theoretical 33 μg per gram. The figures agree quite well with the microbiological data (Table I), only the values for sample No. 3, the white flour, being appreciably greater.

The results obtained by the Bina-Thomas-Brown (1941) procedure were not listed, the values varying from 25% to 47% of the average figures listed in Table II. The low values may be attributed to the inherent inaccuracy of this chemical method. It has been shown (Melnick, Oser, and Siegel, 1941; Melnick and Field, 1940b) that methods which include addition of the aromatic amine to the blank give erroneously low nicotinic acid values.

On the basis of the reports sent in by the collaborators, standard procedures were drawn up and submitted for trial. The details of

these collaborative methods are described in the Appendix to this paper.

Some collaborators were of the opinion that the ubiquitous, but nutritionally inactive, betaine of nicotinic acid, trigonelline, is converted during alkaline extraction into a compound capable of stimulating the growth of the microorganism. Trigonelline is resistant to acid hydrolysis but can be hydrolyzed by alkali (Melnick and Field, 1940a; Melnick, Robinson, and Field, 1940) under certain conditions to yield a pyridine compound which reacts like nicotinic acid in the chemical tests. In order to determine whether trigonelline in cereal products interferes in the microbiological assay for nicotinic acid, tests

TABLE III
NICOTINIC ACID VALUES USING COLLABORATIVE MICROBIOLOGICAL PROCEDURES ¹

Collaborator	Sample 1 (whole wheat flour)			Sample 3 (white flour)			Dried cow-peas		
	Extraction			Extraction			Extraction		
	In H ₂ SO ₄	In NaOH	Enzyme ² + aqueous	In H ₂ SO ₄	In NaOH	Enzyme ² + aqueous	In H ₂ SO ₄	In NaOH	Enzyme ² + aqueous
	<i>μg per gram</i>			<i>μg per gram</i>			<i>μg per gram</i>		
1	59	55	30	8.9	8.6	8.0	18	17	22
2	60	65	44	10.6	9.9	8.4	15	15	21
3	71	69	29	11.4	11.4	6.2	31	31	26
6	57	62	—	10.0	10.0	—	34	23	—
7	59	59	—	8.9	8.8	—	29	17	—
8	57	54	29	9.8	8.5	4.1	20	17	25
9	64	62	—	10.0	9.8	—	26	26	—
11	61	55	43	8.9	8.6	6.3	23	24	18
16	—	68	—	—	—	—	—	25	—
Average	61	61	35	9.8	9.5	6.6	25	22	22

¹ Based upon the method of Snell and Wright.

² Papain + takadiastase digestion

were conducted on both acid and alkaline extracts. For the same reason another product, dried cow-pea meal, was included in the collaborative study. This material is relatively rich in trigonelline (Barger, 1914). The results obtained using the suggested extraction procedures are listed in Table III. The following observations are worth emphasis:

In the microbiological method of assay of cereal products, acid extraction yielded the same nicotinic acid figures as alkaline extraction. In the tests conducted with the cow-pea meal, alkaline extraction definitely failed to yield larger values. These tests constitute good proof that trigonelline does not interfere in the microbiological assays. Enzymic digestion of the sample followed by aqueous extraction

yielded significantly smaller values for nicotinic acid in cereal products. The reproducibility of the microbiological method was good in testing the cereal products but poor in the assay of the cow-pea meal.

Attempts were made to shorten the over-all time of the microbiological method by using a 24-hour rather than a 72-hour incubation period. The results were not satisfactory and therefore are not listed in Table III. The quantity of acid produced during the first 24-hour period was considerably less than that formed during the 72-hour period. Also, consistency among the assay levels in any one test was not good.

Five investigators reported results with the collaborative chemical method. Because of the susceptibility of trigonelline to alkaline hydrolysis, only acid hydrolysis of the samples was carried out. The results, listed in Table IV, show fairly good reproducibility.

TABLE IV
NICOTINIC ACID VALUES USING COLLABORATIVE CHEMICAL PROCEDURE

Collaborator	Sample No. 1 (whole wheat flour)	Sample No. 3 (white flour)	Dried cow-peas
<i>micrograms per gram</i>			
No. 6	73	15.0	29
No. 7	56	11.9	23
No. 8	61	11.5	27
No. 10	53	8.9	21
No. 15	65	10.3	23
Average	62	11.5	25

Additional tests, summarized in Table V, were conducted with samples 1, 3, 8, and 9 by the collaborative chemical method and a number of modifications of it. In all cases good recoveries of added nicotinic acid were obtained. Exhaustive aqueous extraction was found capable of extracting all of the nicotinic acid from these cereal products. It must be remembered that in these experiments the concentrated extracts were subjected to strong acid hydrolysis, whereas in the microbiological method the aqueous extracts were tested directly. In the collaborative method the importance of adjusting the pH of the final test solution to 7.0 is brought out in the tabulated results. Whereas good recoveries of added nicotinic acid were also obtained in the series in which the pH of the final test solutions was adjusted to 4.5, the initial values were somewhat greater, especially in the case of the white flour. It may be that at pH 7.0 the reaction is more specific for nicotinic acid.

Furfural has been found to react with the reagents (Ashford and Clark, 1939) to give a pink color which absorbs light nonspecifically at 420 $m\mu$, the wave length at which the yellow color due to reacted

TABLE V
NICOTINIC ACID VALUES USING COLLABORATIVE CHEMICAL METHOD
AND MODIFICATIONS

Procedure	Nicotinic acid	Sample 1 (whole wheat flour)	Sample 3 (white flour)	Sample 8 (whole wheat bread)	Sample 9 (enriched bread)
Collaborative	Initial ¹	61	<i>micrograms per gram</i>		
	Added Total ¹	70 144	12.2 30.0 40.0	60 70 130	41 50 85
Collaborative but on exhaustive aqueous extract concentrated	Initial ¹	61	10.8	62	45
	Added Total ¹	70 125	15.0 25.8	70 126	45 85
Collaborative but pH of final test solution = 4.5	Initial ¹	68	22.4	64	47
	Added Total ¹	70 133	30.0 52.0	70 123	50 94

¹ Found by chemical analyses.

nicotinic acid absorbs maximally. We have confirmed this observation and investigated it further. Whole wheat flour may contain as much as 8.6% pentosans, most of it localized in the bran (Bailey, 1925). During the initial acid hydrolysis of wheat samples, in the chemical procedure for the determination of nicotinic acid, furfural is formed. The photometric density at 420 m μ due to reacted furfural was found to be only 1/200 of that for nicotinic acid. Moreover, fully 95% of the furfural in the initial hydrolysate is not present in the final test solution. Thus, chemical analyses of the acid-hydrolyzed whole wheat flour (sample No. 1) for both furfural (Stillings and Browning, 1940) and nicotinic acid showed that the maximal error due to the presence of furfural was 7% of the nicotinic acid value. The pentosans do not interfere noticeably in the method involving initial aqueous extraction (Melnick, Oser, and Siegel, 1941), probably because of their relative insolubility. The fact that essentially the same nicotinic acid values are obtained for the four wheat samples following direct acid hydrolysis of the concentrated aqueous extracts (Table V) is additional evidence of the minimal interference of furfural in the collaborative chemical method. Tests made with nicotinic acid and furfural added to solutions buffered⁶ at pH 4.5 and 7.0 indicated that interference due to the presence of furfural is 75% greater when the colorimetric tests are conducted at the lower pH, causing significantly higher nicotinic acid values which are obviously in error.

⁶ To the phosphate buffer used in the chemical method for the determination of nicotinic acid concentrated sodium hydroxide solution was added to adjust the pH, and the resulting solutions used in the above tests.

A comparison of the values obtained by the collaborative chemical and microbiological assay procedures is presented in Table VI. The

TABLE VI
COMPARISON OF NICOTINIC ACID VALUES USING COLLABORATIVE CHEMICAL
AND MICROBIOLOGICAL ASSAY PROCEDURES

No. of collaborators	Procedure	Hydrolysis of sample	Sample 1 (whole wheat flour)	Sample 3 (white flour)	Dried cow peas
			<i>micrograms per gram</i>		
5	Collaborative chemical	Acid	62 (13) ¹	11.5 (18) ¹	25 (14) ¹
8	Collaborative microbiological	Acid	61 (12)	9.8 (12)	25 (12)
9	(Snell-Wright)	Alkali	61 (14)	9.5 (13)	22 (14)
1	Microbiological	Acid	69 (6)	13.1 (7)	—
1	(Dorfman-Koser)	Alkali	71 (3)	12.0 (4)	—

¹ Average values are listed. The figures in parentheses represent number of individual analyses reported using the above procedures

values obtained by five collaborators using the chemical method, by nine collaborators using the Snell-Wright microbiological method, and by one collaborator using the Dorfman-Koser microbiological method are listed. Only the results obtained following acid and alkaline hydrolysis of the samples are compared. These indicate that the values are in good agreement with each other, an excellent index of the specificity of all three methods for nicotinic acid. The values for the cereal products by the chemical method fall between those obtained by the two microbiological procedures.

One point still requires further study before a method can be proposed without reservation. It has been pointed out that, in the microbiological assay, mild aqueous extraction, as such or following enzymic digestion of the samples, yields smaller nicotinic acid values than those obtained when the acid or alkaline extracts are tested. In a report from our laboratory (Oser, Melnick, and Siegel, 1941) it was shown that the increase in nicotinic acid following alkaline extraction is due to a hydrolysis of a compound, otherwise not available to the micro-organism, and not to any greater efficiency in extraction. The pertinent data are reproduced in Table VII. The tests were conducted on three dietary mixtures. When the aqueous extracts were hydrolyzed with alkali of the same normality used for the direct extraction of the samples, essentially the same values were obtained, the figures being significantly greater than those for the initial aqueous extracts. Since the microbiological method can determine nicotinamide, nicotinuric acid, and the nicotinamide-containing coenzymes without preliminary hydrolysis (Snell and Wright, 1941), the material respon-

TABLE VII

INDICATION THAT ALKALINE HYDROLYSIS RATHER THAN EXTRACTION IS RESPONSIBLE FOR INCREASED NICOTINIC ACID VALUES BY MICROBIOLOGICAL METHOD¹

Diet No. ²	Preparation of sample	Nicotinic acid found	Increase in nicotinic acid value
		mg/3000 cal	%
30	(a) Pooled <i>aqueous</i> extract and washings after autoclaving	10.2	—
	(b) Pooled 2 <i>N</i> NaOH extract and washings after autoclaving	13.0	27
	(c) Aqueous extract, as in (a) autoclaved at 2 <i>N</i> alkalinity	13.3	30
31	As (a) above	10.0	—
	As (b) above	12.3	23
	As (c) above	13.4	34
29	As (a) above	7.8	—
	As (c) above	9.9	27

¹ Procedure of Snell and Wright

² These were composites of foods prepared by R. R. Williams and represented average American diets.

sible for the apparent greater nicotinic acid values is not one of the above naturally occurring nicotinic acid derivatives.

The evidence that cereal products contain this material, presumably a nicotinic acid derivative which becomes available to the micro-organism only after hydrolysis, is presented in Table VIII. The values

TABLE VIII

PRESENCE OF AN UNKNOWN NICOTINIC ACID DERIVATIVE IN CEREAL PRODUCTS

Procedure	Sample 1 (whole wheat flour)	Sample 3 (white flour)	Sample 8 (whole wheat flour)	Sample 9 (enriched bread)
	micrograms nicotinic acid per gram			
Mild aqueous extraction	30	5	30	39
Enzyme extraction at pH 4.5	35	7	35	40
Exhaustive aqueous extraction	50	8	50	42
Alkali extraction	62	10	62	43
Acid extraction	61	10	61	45
Aqueous extract subjected to alkali hydrolysis	65	10	65	44
Aqueous extract subjected to acid hydrolysis	—	—	56	—
Incubation of aqueous suspension at pH 8.0.	69	9	—	—
Incubation of aqueous suspension at pH 1.0.	65	11	—	—
Mild aqueous extract autoclaved	44	—	44	—

The above values are not, for the most part, those obtained by specific analyses but are estimated from the voluminous data reported by collaborators 2, 3, 5, 6, 7, 8, and 9, using the Snell-Wright (1941) microbiological assay procedure.

listed are not, for the most part, those obtained by specific analyses but are estimated from the voluminous data reported by seven of the collaborators. Mild aqueous extraction yielded minimal values for nicotinic acid in the collaborative samples but still was capable of extracting all of the nicotinic acid added to enrich the white bread. Enzymic digestion at pH 4.5, followed by mild aqueous extraction, gave slightly greater values. Exhaustive aqueous extraction was capable of yielding much greater nicotinic acid figures approximating the maximal and constant values yielded by acid and alkaline extraction. That the latter values were due to hydrolysis and not to more efficient extraction was indicated by taking mild aqueous extracts and subjecting them to alkaline and acid hydrolysis and obtaining thereby the maximal nicotinic acid values. The unknown nicotinic acid derivative is exceedingly labile since incubation of the aqueous suspension at 37°C at pH 8.0 or pH 1.0, or autoclaving a mild aqueous extract, is sufficient to convert the compound to microbiologically active nicotinic acid.

It is impossible to state at the present time exactly how much of the total nicotinic acid in a cereal product is the unknown nicotinic acid derivative. Because of the ready hydrolysis of the compound, it is conceivable that some conversion to nicotinic acid occurs even in mild aqueous extracts during the subsequent sterilization (autoclaving) of the test solutions prior to inoculation with the microorganism.

Cheldelin and Williams (1942) have found this unknown nicotinic acid derivative to be present in cereal but not in meat products. Whether or not this compound in the unhydrolyzed form is nutritionally available to the mammalian species is still a moot question. Attempts to separate it from the known biologically active derivatives of nicotinic acid are being made by several of the collaborators in order to determine through biological assay whether or not the compound in the unhydrolyzed form is available. The little evidence, thus far accumulated, suggests that it is. A black-tongue dog assay conducted on a rice bran concentrate gave a value of 1.65 mg of nicotinic acid per gram (Waisman, Mickelsen, McKibbin, and Elvehjem, 1940), while the microbiological assay values were 0.83 mg prior to and 1.65 mg after alkaline hydrolysis.⁷ The material is very readily hydrolyzed. Simple incubation of the whole wheat and white flours at pH 1.0 or 8.0, pH values common to the human gastrointestinal tract, converts the compound to a form available for the growth of the microorganism *L. arabinosus*.⁸

⁷ K. Morgareidge, personal communication.

⁸ H. Isbell, personal communication.

In any case both chemical and microbiological methods are able to determine added nicotinic acid with a good degree of accuracy. For this reason, despite the unsettled question of the nutritional value of the unknown nicotinic acid derivative in cereal products, both methods should prove valuable for controlling the enrichment of flour and bread with nicotinic acid.

Appendix

COLLABORATIVE PROCEDURES FOR THE PREPARATION OF EXTRACTS FOR THE MICROBIOLOGICAL ASSAY OF CEREAL PRODUCTS FOR NICOTINIC ACID

Alkaline Extraction

For nonenriched white flour: A two-gram sample is suspended in 50 ml of water, 50 ml of 2*N* sodium hydroxide solution are added, and the suspension is autoclaved at 15 lbs pressure¹ for 30 minutes. The hot suspension is centrifuged and washed twice with 50 ml of boiling water. The "extract" and washings are pooled, neutralized with concentrated hydrochloric acid to pH 7.0 (litmus as the external indicator) and the volume brought to 250 ml.¹

For whole wheat flour: A one-gram sample is extracted (and hydrolyzed) with 100 ml of 1*N* sodium hydroxide. The final volume is brought to 500 ml.¹

For other products: By taking appropriate-size samples or by proper dilutions, other cereal products such as enriched flour, enriched bread, bran, etc. may be "extracted" to yield solutions in the proper testing range.¹

Acid Extraction

Sulfuric acid of the same normality may be used for the extraction in place of the above sodium hydroxide. The neutralization is then made with sodium hydroxide.¹

Enzymic Digestion Followed by Mild Aqueous Extraction

To a two-gram sample 20 ml of 0.1% acetate buffer at pH 4.6 are added. To the suspension a paste containing takadiastase and papain is added to yield 20 mg of each of the enzyme preparations. The digestion is allowed to proceed for 6 to 8 hours at 48° to 50°C under benzene. The material is steamed for 30 minutes to remove the benzene and to destroy the enzymes, filtered and diluted to appropriate volume. The values obtained by the enzymic digestion procedure will be considerably less than those obtained by the two methods above.

Microbiological Assay

The reference curve is evolved according to the published procedure of Snell and Wright (1941) with slight modifications. If charcoal-treated casein hydrolysates are used p-aminobenzoic acid should be added to the medium, 1 microgram per 10 ml (Isbell, 1942). The medium should contain 1% glucose. The inoculated samples are incubated at 37°C for 72 hours.

COLLABORATIVE CHEMICAL METHOD FOR THE DETERMINATION OF NICOTINIC ACID IN CEREAL PRODUCTS

Based upon the procedures of Melnick and Field (1940a, b), Dann and Handler (1941), Cannon² and Gortner³

Reagents

Cyanogen bromide: Water saturated with bromine at 5° to 10°C is just decolorized in the cold by the addition of a 10% potassium cyanide solution. From 70 to 75 ml of the potassium cyanide solution is used in the titration of 500 ml of bromine water. This reagent, when stored in the refrigerator at about 5°C, will keep almost indefi-

¹ Further studies have shown that it is unnecessary to separate the extract from the insoluble residue. The extracted suspension after neutralization may be tested directly.

² H. J. Cannon, personal communication.

³ W. A. Gortner, personal communication.

nately (more than 5 months). A much simpler, but satisfactory, procedure involves simple solution of cyanogen bromide crystals (Eastman Kodak Co., Rochester, N. Y.) in cold water to 4 per cent concentration. The crystals should also be stored in the refrigerator.

Aniline solution: Redistilled aniline is dissolved in absolute ethyl alcohol to make 4% solution. When stored in a brown glass bottle at room temperature, this reagent will keep for months.

Standard nicotinic acid solution: 10.0 mg of nicotinic acid per 100 ml of absolute ethyl alcohol. This solution will keep indefinitely.

Buffer solution:⁴ Composed of 1960 ml of water, 30 ml of 15% sodium hydroxide solution, 8 ml of phosphoric acid (85%) and 333 ml of absolute ethyl alcohol.

Hydrochloric acid solution: Concentrated, specific gravity about 1.18.

Sulfuric acid solution: 0.20*N*.

Phosphoric acid solution: A 20% solution.

Sodium hydroxide solution: Concentrated (approximately 18 normal) and 0.50*N*.

Phenolphthalein solution: One % in alcohol.

Methyl violet solution: 0.1% in water.

Lead nitrate.

Tertiary potassium phosphate

Lloyd's reagent.⁵

Hydrolysis, Adsorption, Elution, and Decolorization

A sample containing from 20 to 100 μ g of nicotinic acid is weighed into a test tube calibrated at 15.0 ml and 16.5 ml. Ten ml of water and 5 ml of concentrated hydrochloric acid are added. The tube is immersed in a boiling-water bath and the hydrolysis allowed to proceed for 30 to 40 minutes with occasional stirring. The sample is cooled to room temperature and the pH adjusted to 0.5 to 1.0 with 18*N* NaOH (approximately 3 ml are required) using methyl violet as the external indicator.⁶ Two and one-half grams of Lloyd's reagent are added and the suspension shaken vigorously for about one minute. After centrifugation, the supernatant liquid is discarded and the residue washed twice with 10 ml of the 0.20*N* sulfuric acid, care being taken to completely disintegrate it by vigorously shaking at each washing. The suspensions are centrifuged and the supernatants discarded. To the washed residue 12 ml of 0.5*N* sodium hydroxide are added and the mixture shaken vigorously for one minute. Water is then added to bring the volume to the 16.5-ml mark. The volume of solution is 15.0 ml. The tube is again shaken and the resultant suspension centrifuged. The eluate is drained as completely as possible into a clean dry test tube containing 0.7 g of finely powdered lead nitrate.

After shaking for one minute the suspension is centrifuged and the decolorized solution transferred to another clean, dry test tube. One drop of phenolphthalein is added, followed by solid potassium phosphate until the color of the solution becomes slightly pink. One to two drops of 20% phosphoric acid solution is added from a capillary pipette until the solution is neutral to litmus, the latter being used as an external indicator. The suspension is centrifuged. The clear supernatant solution is drawn by oral suction through a U-tipped pipette into a test tube, accurately graduated in 0.1-ml quantities to 15 ml.

Color Development, Readings and Calculations

The volume of the collected supernatant solution is noted and then brought to the 10-ml mark. For the colorimetric tests 3-ml portions of the test solution are used. (a) To the first sample 7 ml of the alcoholic buffer solution is added. (b) To the second aliquot, 6 ml of the cyanogen bromide reagent is added from a burette, followed in exactly 10 minutes with 1 ml of the aniline reagent. The solutions are stirred after the addition of each reagent. (c) To the third aliquot 0.1 ml of the standard nicotinic acid solution is added, followed by the reagents as in (b).

The yellow color developed in solutions (b) and (c) is read after five minutes⁷ in a photoelectric colorimeter, using a 420-m μ filter. By subtracting from the photo-

⁴ With phenolphthalein as the indicator, 7 ml of the solution have a titratable acidity equivalent to 9.4 ml of 0.05*N* NaOH; its pH is 3.3. These values correspond to the titratable acidity and pH of the pooled reagents 6 ml of CNBr plus 1 ml of C₆H₅NH₂, determined under conditions the same as those observed in the reaction with nicotinic acid.

⁵ A form of hydrated aluminum silicate, obtained from the Eli Lilly & Co., Indianapolis, Ind.

⁶ This is conveniently carried out on a spot plate using as a control methyl violet added to 0.2*N* sulfuric acid.

⁷ This corresponds to the maximal color intensity. It reaches a maximum in 3 minutes and is constant for the next 5 minutes.

metric density of solution (b) that of solution (a) due to the color remaining after decolorization, the photometric density of the reacted nicotinic acid is obtained. This is converted into absolute units of nicotinic acid by correlating the increment in photometric density, solution (c) minus solution (b), with the amount of nicotinic acid added.

In the colorimetric measurements it is necessary to have two center settings, one for evaluating the residual color in the test solution, the other for the color developed by the chemical reaction. The photoelectric colorimeter is set to give a galvanometer reading of 100.0 (zero photometric density) with a solution containing 3 ml of water, and 7 ml of alcoholic buffer solution. Using the resulting center setting (No. 1, galvanometer reading with the test tube or cuvette containing the pure solution now removed), the blank solutions are read in turn to determine the residual color of each of the test solutions (blank). The colorimeter is then set to give a galvanometer reading of 100.0 with a solution containing 3 ml of water, 6 ml of cyanogen bromide reagent, and 1 ml of aniline solution. All the subsequent solutions containing reacted nicotinic acid are read, using the new center setting (No. 2). Galvanometer readings are converted into photometric density by the formula

$$L = 2 - \log G,$$

where L = photometric density and G = galvanometer reading. (Most instruments have a dual scale which allows direct reading in terms of photometric density; others are supplied with a conversion table.)

If the colorimeter cell requires more than 10 ml (and less than 20 ml) of solution, the decolorized, neutralized test solutions are diluted to double volume with water and 6-ml aliquots are used for testing. Double quantities of reagents are also used. If in place of the test-tube type of cell, a flat cuvette is used, the center settings will be greater than 100.0 and therefore will be off the scale. In such cases the instrument is set to give a galvanometer reading of 50.0 instead of 100.0 and all readings are multiplied by 2 to give the correct value for conversion to photometric density.

Typical Calculations

I. Test substance = 1.000 g of whole-wheat flour.

Eight ml of the final neutralized supernatant solution were diluted to 10 ml, and 3-ml aliquots were taken for testing.

Colorimeter cell = test tube

Volume of the solutions to be read = 10 ml

Center-setting (No. 1) = 73.5 galvanometer reading

Center-setting (No. 2) = 89.0 " "

Solution (a)

using center-setting (No. 1) = 90.0 galvanometer reading
= 0.046 photometric density

Solution (b)

using center-setting (No. 2) = 36.5 galvanometer reading
= 0.438 photometric density

Solution (c)

using center-setting (No. 2) = 15.0 galvanometer reading
= 0.824 photometric density

$0.438 - 0.046 = 0.392$ photometric density due to reacted nicotinic acid

$0.824 - 0.438 = 0.386$ photometric density due to reacted nicotinic acid

$0.392/0.386 \times 10 = 10.2$ μ g of nicotinic acid in 3-ml aliquot of the test solution

$10.2 \times 10/3 \times 15/8 = 63.8$ μ g of nicotinic acid per gram of whole wheat flour

II. Same test solution as the above, but the colorimeter cell = flat cuvette and volume of solutions to be read = 20 ml. The 10-ml test solution is diluted with water to 20 ml and 6-ml aliquots are taken for the analyses.

Center-setting (No. 1) = 54.0 galvanometer reading (observed)

= 107.0 galvanometer reading (corrected)

Center-setting (No. 2) = 67.0 galvanometer reading (observed)

= 134.0 galvanometer reading (corrected)

Solution (a)

using center-setting (No. 1) = 45.0 galvanometer reading (observed)
= 90.0 galvanometer reading (corrected)
= 0.046 photometric density

Solution (b)
using center-setting (No. 2) = 18.25 galvanometer reading (observed)
= 36.5 galvanometer reading (corrected)
= 0.438 photometric density

Solution (c)
using center-setting (No. 2) = 7.5 galvanometer reading (observed)
= 15.0 galvanometer reading (corrected)
= 0.824 photometric density

$0.438 - 0.046 = 0.392$ photometric density due to reacted nicotinic acid
 $0.824 - 0.438 = 0.386$ photometric density due to $10\ \mu\text{g}$ of reacted nicotinic acid
 $0.392/0.386 \times 10 = 10.2\ \mu\text{g}$ of nicotinic acid in 6 ml aliquot of the test solution
 $10.2 \times 20/6 \times 15/8 = 63.8\ \mu\text{g}$ of nicotinic acid per gram of whole wheat flour

Summary

Microbiological assays conducted on acid and alkaline extracts of cereal products yield reproducible, maximal values for nicotinic acid.

Aqueous extraction, without preliminary enzymic digestion, removes all the nicotinic acid from cereal products but yields, according to microbiological assays, smaller nicotinic acid values than those obtained for the acid or alkaline extracts.

The apparently greater nicotinic acid content of the acid or alkaline extracts is not due to a greater extraction efficiency but to hydrolysis of an unknown compound, which otherwise is unavailable to the microorganism.

The unknown compound, presumably a nicotinic acid derivative, is believed to be biologically available to man but definite proof of this must await biological assay.

The unknown compound is undoubtedly included in the values obtained by chemical assay since all chemical procedures involve preliminary hydrolysis of the samples (or extracts).

Collaborative microbiological and chemical procedures are described and the results of a collaborative study using these methods are presented.

The collaborative procedures yield reproducible values, good recoveries of nicotinic acid, and are specific for the vitamin in the assay of cereal products.

Furfural, produced from pentosans in wheat products, reacts with the reagents in the chemical procedure for the determination of nicotinic acid. However, in the collaborative method, interference due to the presence of furfural in the final test solution is negligible.

No loss of nicotinic acid occurs in the production of bread from whole wheat flour or nicotinic acid-enriched white flour.

Acknowledgments

The author wishes to express his gratitude to the collaborators who served on the Nicotinic Acid Committee and to his colleagues Dr. Bernard L. Oser, Mr. Louis Siegel, and Mr. Norman Wiederhorn of Food Research Laboratories for their generous cooperation in the present study.

Literature Cited

- Ashford, W. R., and Clark, R. H.
1939 Comparison of colorimetric methods for the determination of nicotinic acid. *Trans. Roy. Soc. Can III*, **33**: 29-32.
- Bailey, C. H.
1925 Chemistry of wheat flour. *Am. Chem. Soc. Monograph Series*.
- Barger, G.
1914 The simpler natural bases. *Monographs on Biochemistry*. London and N. Y.
- Bina, A. F., Thomas, J., and Brown, E. B.
1941 The determination of nicotinic acid in bread and other cereal products. *Cereal Chem.* **18**: 661-666.
- Cheldelin, F., and Williams, R. R.
1942 Comparison of methods of extraction and assay of nicotinic acid from animal and plant tissues. *Ind. Eng. Chem. (Anal. Ed.)* (in press).
- Dann, W. J., and Handler, P.
1941 The quantitative estimation of nicotinic acid in animal tissues. *J. Biol. Chem.* **140**: 201-213.
- Dorfman, A., Koser, S. A., Horwitt, M. K., Berkman, S., and Saunders, F.
1940 Quantitative response of the dysentery bacillus to nicotinamide and related compounds. *Proc. Soc. Exp. Biol. Med.* **43**: 434-438.
- Isbell, H.
1942 Effect of p-aminobenzoic acid on the microbiological assay for nicotinic acid. *J. Biol. Chem.* **144**: 567-568.
- Melnick, D., and Field, H.
1940a Determination of nicotinic acid in biological materials by means of photoelectric colorimetry. *J. Biol. Chem.* **134**: 1-16.
1940b Chemical determination of nicotinic acid: inhibitory effect of cyanogen bromide upon the aniline side reactions. *J. Biol. Chem.* **135**: 53-58.
- Oser, B. L., and Siegel, L.
1941 Chemical determination of nicotinic acid content of flour and bread. *Ind. Eng. Chem. (Anal. Ed.)* **13**: 879-883.
- Robinson, W. D., and Field, H., Jr.
1940 Influence of the excretion of other pyridine compounds upon the interpretation of the urinary nicotinic acid values. *J. Biol. Chem.* **136**: 131-144.
- Oser, B. L., Melnick, D., and Siegel, L.
1941 Comparison of microbiological and chemical methods for determination of nicotinic acid with observations on cooking losses. Abstracts, Div. Biol. Chem., *Am. Chem. Soc.*, Atlantic City, N. J.
- Snell, E. E., and Wright, L. D.
1941 A microbiological method for the determination of nicotinic acid. *J. Biol. Chem.* **139**: 675-686.
- Stillings, R. A., and Browning, B. L.
1940 Photocolorimetric determination of furfural. *Ind. Eng. Chem. (Anal. Ed.)* **12**: 499-502.
- Waisman, H. A., Mickelsen, O., McKibbin, J. M., and Elvehjem, C. A.
1940 Nicotinic acid potency of food materials and certain chemical compounds. *J. Nutrition* **19**: 483-492.

THE EFFECT OF CERTAIN INGREDIENTS AND VARIATIONS IN MANIPULATIONS ON THE FARINOGRAPH CURVE

CLAUDE L. MOORE and RALPH S. HERMAN

Washburn Crosby Co., Buffalo, N. Y., Eastern Division General Mills, Inc.

(Read at the Annual Meeting, May 1940)

It has seemed of interest to measure the influence of irregular manipulation and the effects of adding varying amounts of materials, commonly employed in commercial baking practice, upon the Brabender farinograph curves that are characteristically obtained with the usually employed mixture of only flour and water. We have found it convenient to employ a system of tabulating these results that is quite contrary in principle and application to the method commonly followed. This plan of measurement has proved over the past two years to be a simple form of expression that can be readily replicated.

We have selected three terms to designate the three measurements employed: The "initial phase" represents in minutes the period from the start of mixing to the point where the upper side of the curve first touches the 500 consistency line. The "period of resistance" represents the period in minutes measured from the point where the upper side of the curve first touches the 500 consistency line to the point where this same upper side of the curve descends to the 500 consistency line. The "factor X" is plotted by taking one-fourth of the "period of resistance" plus the "initial phase."

This method of measurement has been arbitrarily established, but it is readily applicable to any type of curve and with experience gives a definite picture of flour characteristics as established by the Brabender farinograph. At this time we are offering no suggestions as to the interpretative value of any of these designations beyond the statement that no one of the three measurements, as shown in our data, can be accepted as a single index for scoring flour characteristics.

For the purpose of this study we selected four commercially produced northwestern-type patent flours of the 1939 crop, having previously made a similar study on flour types of the 1938 crop. These flours were carefully blended together in sufficient quantity to provide an adequate supply for the series of tests reported. This flour had an ash content of 0.40%, a protein content of 12.8%, and an absorption of 65.8%. These values were calculated to a 13.5% moisture basis.

Figure 1 represents the normal curve characteristics of this flour blend. We obtained an absorption of 65.8%, an "initial phase" of

2.5 minutes, a "period of resistance" of 22.5 minutes, and a "factor X" of 8.1.

Skovholt and Bailey (1932) have shown that temperature differences cause plasticity variations in doughs, depending upon their stiffness. Brabender and Hartkopf (1938) have recommended that a temperature of 86°F be maintained during manipulation of the farino-

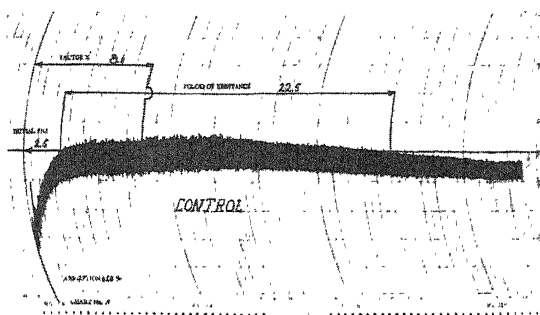


Fig 1 Normal curve characteristics of flour blend showing plan of measurement employed

graph. Temperatures employed in commercial bakery practice vary over a considerable range and the Brabender curve obtained at 86°F is by no means comparable with the curve obtained at other temperatures. The effect of varying temperatures is shown in Table I and Figure 2.

TABLE I
EFFECT OF VARYING TEMPERATURES

Temperature	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	ζ_o	min	min	
76°F	70.6	9.0	18.0	13.5
78°F	69.0	8.0	18.0	12.5
80°F	68.1	7.0	20.0	12.0
82°F	67.2	5.5	19.5	10.4
84°F	66.4	3.5	22.5	9.1
86°F (30°C) ¹	65.8	2.5	22.5	8.1
88°F	64.1	1.5	26.0	8.0
90°F	62.9	1.5	23.0	7.4

¹ Control

It will be observed that as the temperature increases, the "initial phase" decreased, the "period of resistance" increased, and the "factor X" decreased. The consistency of the dough likewise exhibited a very substantial influence on the characteristics of the curve obtained.

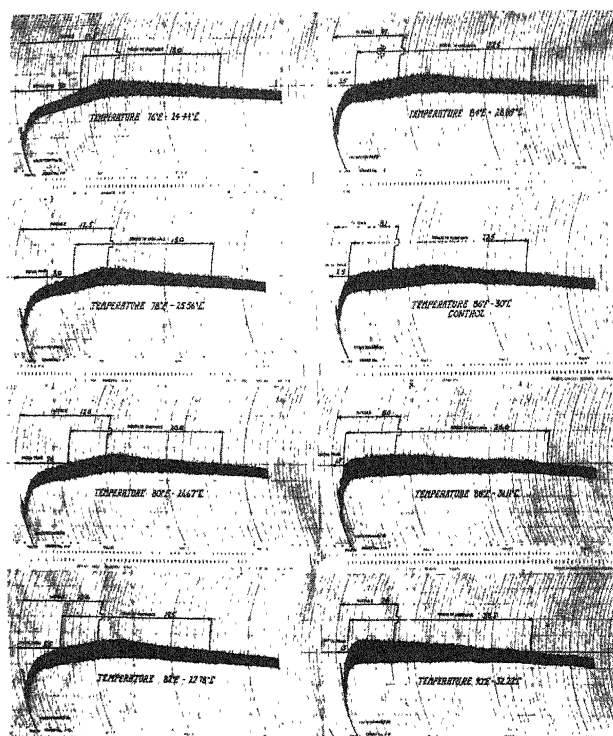


Fig 2. Effect of varying temperatures

In Table II and Figure 3 is shown the effect of varying absorptions designed to produce a curve on a definite consistency line, ranging from a normal 500 Brabender units down to 300 units and up to 800 units, in increments of 100 units.

TABLE II
EFFECT OF VARYING DOUGH CONSISTENCY

Units	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	C_p	<i>min</i>	<i>min</i>	
800	56.0	1.0	15.0	4.7
700	58.8	1.0	17.0	5.2
600	61.8	2.5	18.0	7.0
500 ¹	65.8	2.5	22.5	8.1
400	70.1	12.0	28.0	19.0
300	78.9	32.0	22.0	38.2

¹ Control.

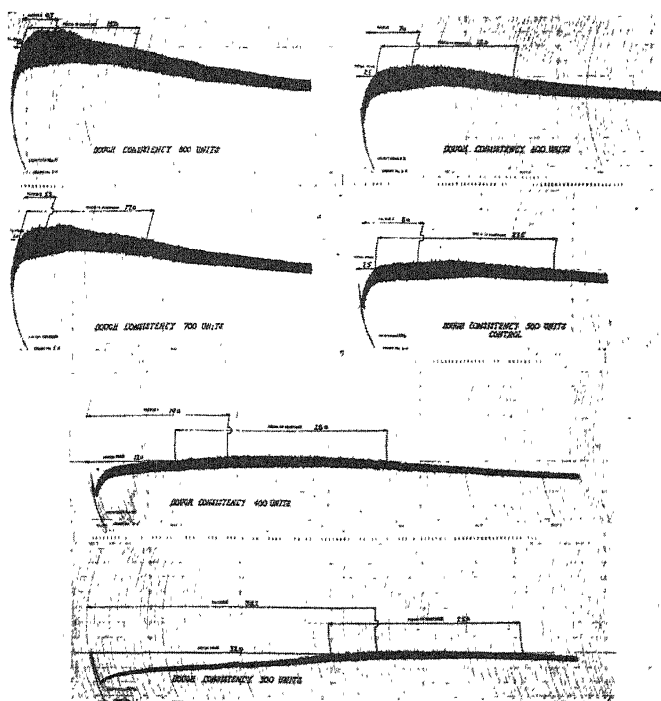


Fig. 3 Effect of varying dough consistency

It will be noted that the absorption varied from 56.0% for an 800-unit-consistency dough to 78.9% for a 300-unit-consistency dough. The initial phase ranged from 1.0 on the softest dough to 32.0 for the stiffest dough. The period of resistance and factor X increased likewise as the absorption increased.

The effect of salt in the baking process is well established. The addition of varying amounts of sodium chloride to a flour has a marked effect upon the type of curve produced. This is shown in Table III and Figure 4.

TABLE III
EFFECT OF VARYING PERCENTAGES OF SODIUM CHLORIDE

Sodium chloride	Absorption, 13 5% mb	Initial phase	Period of resistance	Factor X
	%	min	min	
Control	65.8	2.5	22.5	8.1
1%	65.0	5.0	28.0	12.0
2%	65.4	9.0	27.0	15.7
3%	66.5	13.5	29.5	20.0
4%	67.5	18.0	35.0	26.8

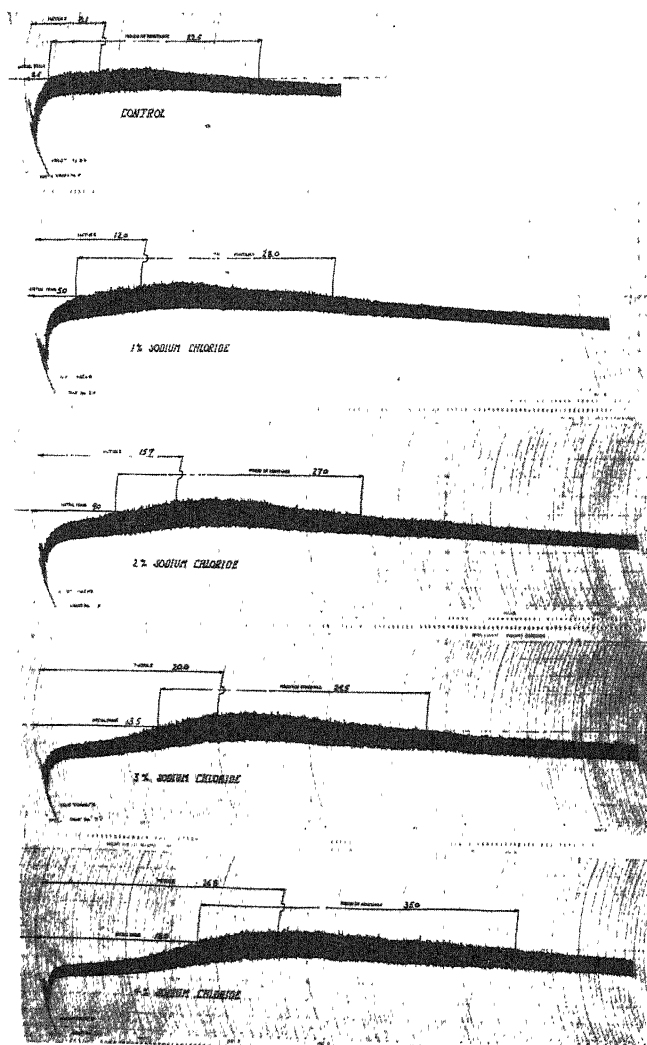


Fig. 4. Effect of varying percentages of sodium chloride

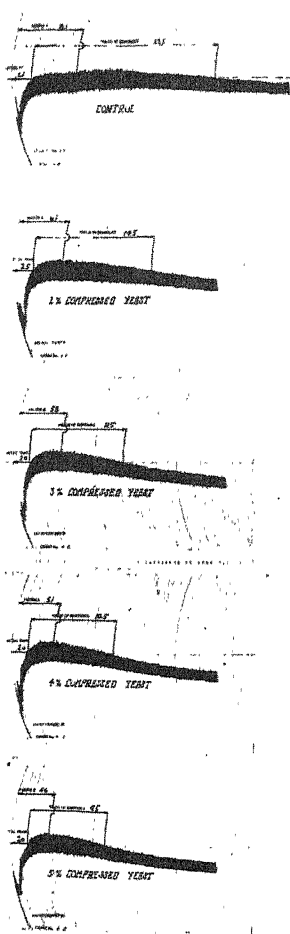


Fig. 5. Effect of varying percentages of compressed yeast

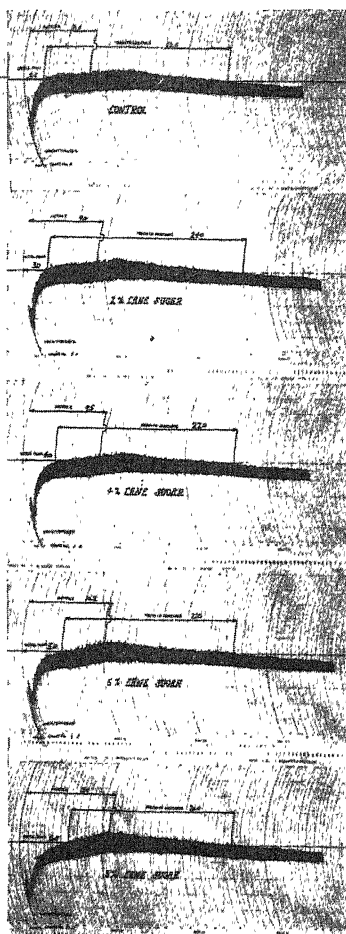


Fig. 6. Effect of varying percentages of cane sugar

The initial phase, the period of resistance, and the factor X were all increased as the percentage of sodium chloride was increased. A factor X, for example, of 8.1 on the control flour was increased to 26.8 with 4% of sodium chloride. The absorption level increased with the higher increments of sodium chloride.

Compressed yeast when added in different amounts to the control flour had little effect upon either the absorption or the initial phase, but

it did vary the period of resistance and factor X. This influence is shown in Table IV and Figure 5.

TABLE IV
EFFECT OF VARYING PERCENTAGES OF COMPRESSED YEAST

Compressed yeast	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	min	min	
Control	65.8	2.5	22.5	8.1
2%	64.7	2.5	14.5	6.1
3%	64.7	2.0	11.5	5.8
4%	64.7	2.0	10.5	5.1
5%	64.7	2.0	9.5	4.6

Cane sugar likewise had an influence on the characteristics of the Brabender farinograph curve, as indicated in Table V and Figure 6. With increased amounts of cane sugar the initial phase increased, the period of resistance decreased, and the factor X increased.

TABLE V
EFFECT OF VARYING PERCENTAGES OF CANE SUGAR

Cane sugar	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	min	min	
Control	65.8	2.5	22.5	8.1
2%	64.5	3.0	24.0	9.0
4%	64.3	4.0	22.0	9.5
6%	64.0	5.0	21.0	10.2
8%	63.6	6.0	20.0	11.0

Corn sugar had a similar effect on the curve, but the degree of change was more pronounced as indicated in Table VI and Figure 7. It will be observed that 4% of corn sugar raised the initial phase from 2.5 in the control to 7.0, as compared with 4.0 for cane sugar. In turn, factor X was increased to 12.0 from 8.1 in the control and 9.5 for cane sugar.

TABLE VI
EFFECT OF VARYING PERCENTAGES OF CORN SUGAR

Corn sugar	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	min	min	
Control	65.8	2.5	22.5	8.1
2%	65.2	4.0	23.0	9.8
4%	65.4	7.0	20.0	12.0
6%	65.7	7.5	20.5	12.7
8%	65.9	8.0	22.0	13.5

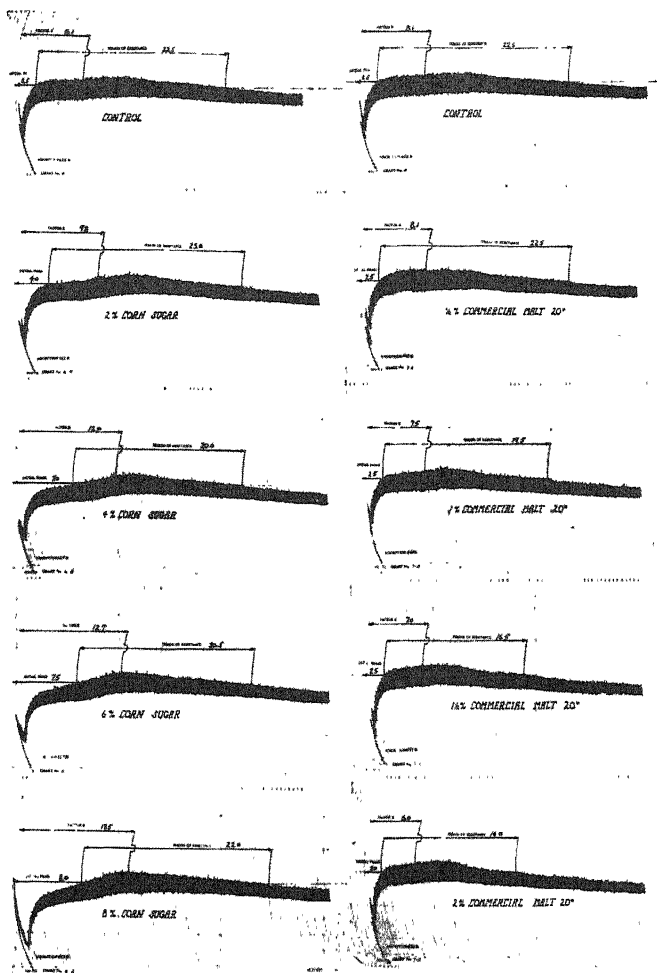


Fig. 7. Effect of varying percentages of corn sugar.

Fig. 8. Effect of varying percentages of commercial malt, 20° Lintner.

The effect of 20°L commercial malt syrup was less pronounced than for either cane sugar or corn sugar. As indicated in Table VII and Figure 8, the period of resistance and factor X showed a slight decrease with increasing increments of 20°L malt added to the flour and water mixture.

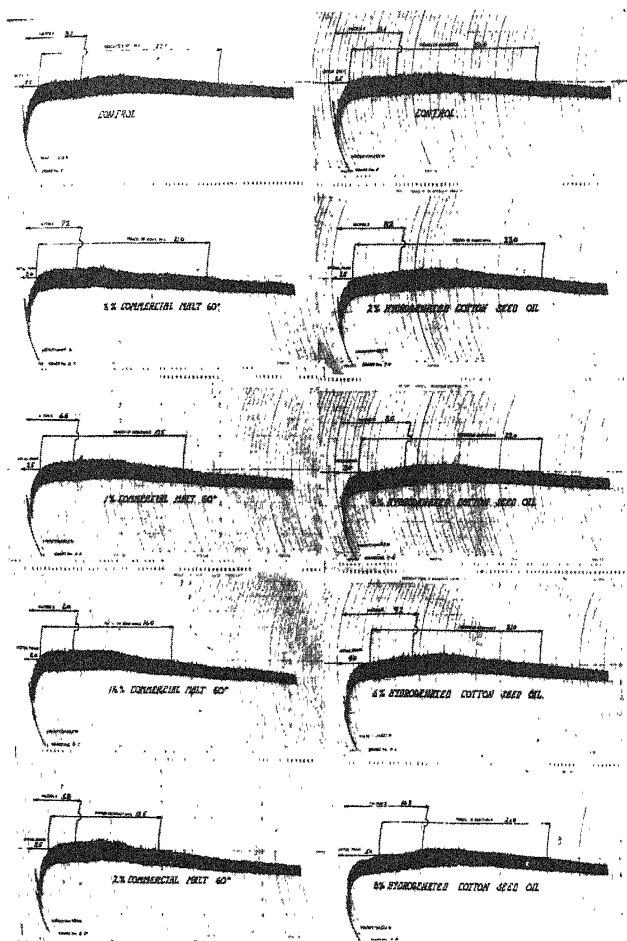


Fig. 9. Effect of varying percentages of commercial malt, 60° Lintner.

Fig. 10. Effect of varying percentages of hydrogenated cottonseed oil.

The degree of variation was slightly more evident with 60°L commercial malt syrup, as shown in Table VIII and Figure 9. In fact, the effect of both types of malt, as would be expected, was quite the reverse of that noted with either cane sugar or corn sugar.

Hydrogenated cottonseed oil in increasing amounts, as indicated in Table IX and Figure 10, raised the initial phase very slightly, like-

TABLE VII

EFFECT OF VARYING PERCENTAGES OF COMMERCIAL MALT, 20° LINTNER

Malt, 20°L	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	min	min	
Control	65.8	2.5	22.5	8.1
½%	64.7	2.5	22.5	8.1
1%	64.4	2.5	19.5	7.5
1½%	63.9	2.5	16.5	7.0
2%	63.6	2.0	16.0	6.0

TABLE VIII

EFFECT OF VARYING PERCENTAGES OF COMMERCIAL MALT, 60° LINTNER

Malt, 60°L	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	min	min	
Control	65.8	2.5	22.5	8.1
½%	64.5	2.0	21.0	7.2
1%	64.2	2.5	17.5	6.6
1½%	63.5	2.0	16.0	6.0
2%	62.8	2.5	13.5	5.8

TABLE IX

EFFECT OF VARYING PERCENTAGES OF HYDROGENATED COTTONSEED OIL

Cottonseed oil	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	min	min	
Control	65.8	2.5	22.5	8.1
2%	63.7	2.5	23.0	8.2
4%	62.9	3.0	22.0	8.5
6%	62.1	4.0	21.0	9.2
8%	62.0	5.0	21.0	10.3

wise the period of resistance, and slightly increased factor X, compared with the control flour.

The effect of dairy butter, as represented in Table X and Figure 11,

TABLE X

EFFECT OF VARYING PERCENTAGES OF DAIRY BUTTER

Dairy butter	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	min	min	
Control	65.8	2.5	22.5	8.1
2%	64.0	2.0	24.0	8.0
4%	63.0	2.0	24.0	8.0
6%	61.2	1.5	26.5	8.1
8%	59.2	1.5	26.0	8.1

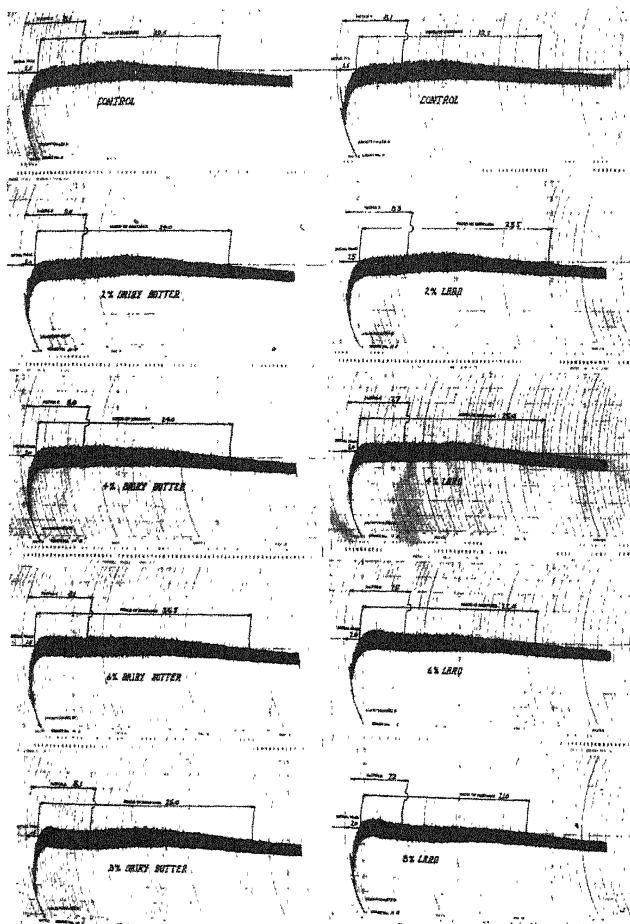


Fig. 11. Effect of varying percentages of dairy butter.

Fig. 12. Effect of varying percentages of leaf lard.

was quite the reverse of the curve characteristics obtained with hydrogenated cottonseed oil. The initial phase was only slightly affected, the factor X was unchanged, and the period of resistance was only slightly advanced with increasing increments of dairy butter. It will be noted, however, that the absorption was lowered from 64% for 2% of dairy butter, to 59.2 when 8% of dairy butter was added to the flour and water mixture.

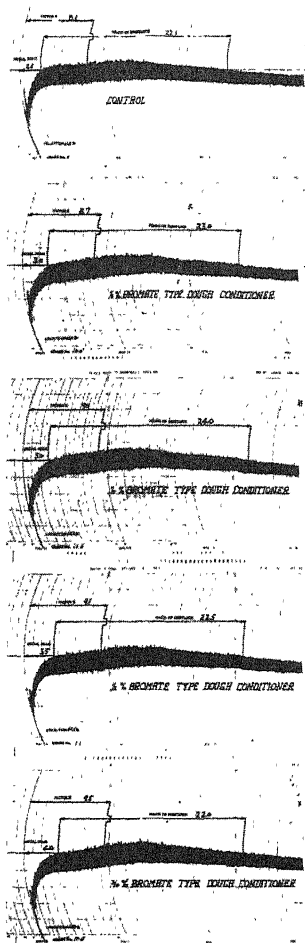


Fig. 13. Effect of varying percentages of bromate-type dough conditioner.

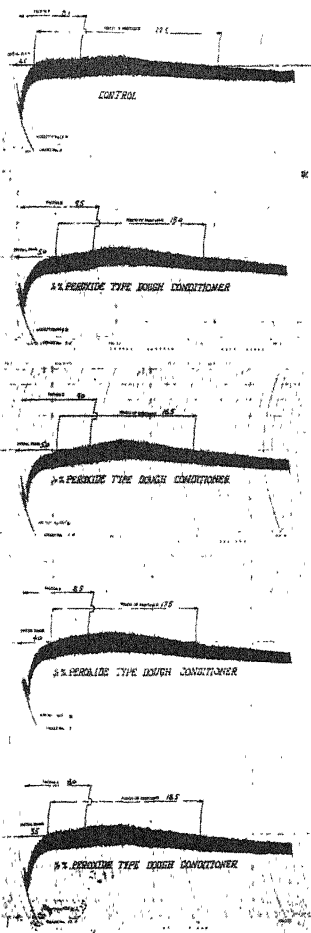


Fig. 14. Effect of varying percentages of peroxide-type dough conditioner.

Leaf lard as shown in Table XI and Figure 12 had much the same reaction as dairy butter. The initial phase and the period of resistance were only very slightly influenced, while "factor X" showed but a minor decrease with increasing increments of leaf lard. The absorption level, however, decreased with increasing amounts of lard in practically the same ratio as with dairy butter.

TABLE XI
EFFECT OF VARYING PERCENTAGES OF LEAF LARD

Leaf lard	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	min	min	
Control	65.8	2.5	22.5	8.1
2%	64.6	2.5	23.5	8.3
4%	63.0	2.0	23.0	7.7
6%	62.2	2.0	22.0	7.5
8%	61.7	2.0	21.0	7.2

Dough conditioners affect the curve to varying degrees. A dough conditioner of the bromate type as represented in Table XII and Figure 13 slightly increased the absorption as the material was added in increasing amounts to the flour-and-water mixture. The initial phase and the factor X were slightly increased in comparison with the control, while the "period of resistance" was only slightly affected.

TABLE XII
EFFECT OF VARYING PERCENTAGES OF BROMATE-TYPE DOUGH CONDITIONER

Bromate conditioner	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	min	min	
Control	65.8	2.5	22.5	8.1
1/8%	65.0	3.0	23.0	8.7
1/4%	65.2	3.0	24.0	9.0
1/2%	65.5	3.5	22.5	9.1
3/4%	65.8	4.0	22.0	9.5

A dough conditioner of the peroxide type, as represented in Table XIII and Figure 14, had a more pronounced effect, particularly in increasing the absorption level. It is interesting to note in this instance that one-eighth of 1% dosage of this material affected the initial phase and the factor X to a greater degree than did larger increments.

TABLE XIII
EFFECT OF VARYING PERCENTAGES OF PEROXIDE-TYPE DOUGH CONDITIONER

Peroxide conditioner	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	min	min	
Control	65.8	2.5	22.5	8.1
1/8%	66.6	5.0	18.0	9.5
1/4%	66.9	5.0	16.5	9.0
1/2%	67.2	4.0	17.5	8.5
3/4%	67.6	3.5	18.5	8.0

A dough conditioner of the iodate and phosphate type, on the other hand, while showing practically no variation in absorption materially

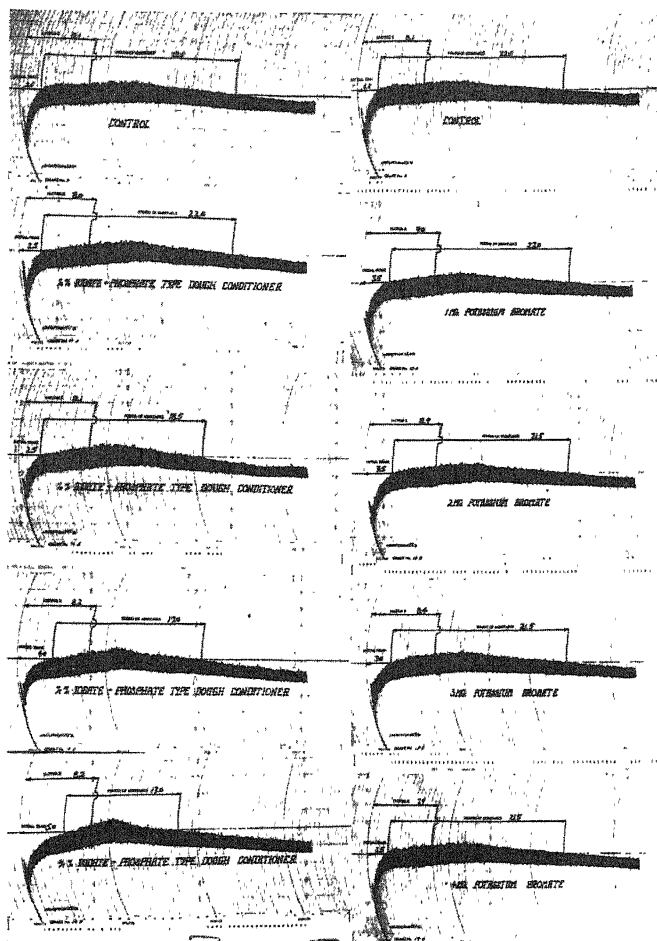


Fig 15. Effect of varying percentages of iodate-type dough conditioner.

Fig 16. Effect of varying amounts of potassium bromate.

increased the initial phase and reduced the period of resistance as increasing dosages were added to the flour-and-water mixture. This is shown in Table XIV and Figure 15.

The effect of potassium bromate by itself is not especially different from the results obtained in using the bromate-type dough conditioner. As represented by Table XV and Figure 16, increased increments of potassium bromate had practically no effect upon the absorption level,

TABLE XIV
EFFECT OF VARYING PERCENTAGES OF IODATE- AND PHOSPHATE-TYPE
DOUGH CONDITIONER

Conditioner	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	<i>%</i>	<i>min</i>	<i>min</i>	
Control	65.8	2.5	22.5	8.1
1/8%	65.1	2.5	22.0	8.0
1/4%	65.3	2.5	18.5	8.1
1/2%	65.5	4.0	17.0	8.2
3/4%	65.8	5.0	13.0	8.2

TABLE XV
EFFECT OF VARYING AMOUNTS OF POTASSIUM BROMATE

Potassium bromate	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	<i>%</i>	<i>min</i>	<i>min</i>	
Control	65.8	2.5	22.5	8.1
1 mg	65.4	3.5	22.0	9.0
2 mg	65.3	3.5	21.5	8.9
3 mg	65.2	3.0	21.5	8.4
4 mg	65.1	2.5	21.5	7.9

while the initial phase, the period of resistance, and the factor X were all slightly decreased.

Skovholt and Bailey (1932) reported on the plasticity characteristics of a dough containing 6% of dry skim milk with and without the further addition of 1.75% of sodium chloride. We have found that dry skim-milk powder, when added to the flour and water mixture, has a pronounced effect upon the "initial phase," the "period of resistance," and the "factor X" as increased percentages of the dry skim milk were employed. This effect is shown in Table XVI and Figure 17.

TABLE XVI
EFFECT OF VARYING PERCENTAGES OF DRY SKIM-MILK POWDER

Dry skim-milk powder	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	<i>%</i>	<i>min</i>	<i>min</i>	
Control	65.8	2.5	22.5	8.1
2%	65.5	3.5	26.5	10.0
4%	67.1	7.0	24.0	13.0
6%	69.6	10.0	29.0	17.2
8%	71.8	11.5	30.5	19.0

It will be noted, also, that the absorption level increased from 65.5 with 2% of dry skim-milk powder, to 71.8 with 8% of dry skim-milk powder, while the initial phase increased to 11.5 and the period of resistance to 30.5, as compared with the initial phase of 2.5 and the

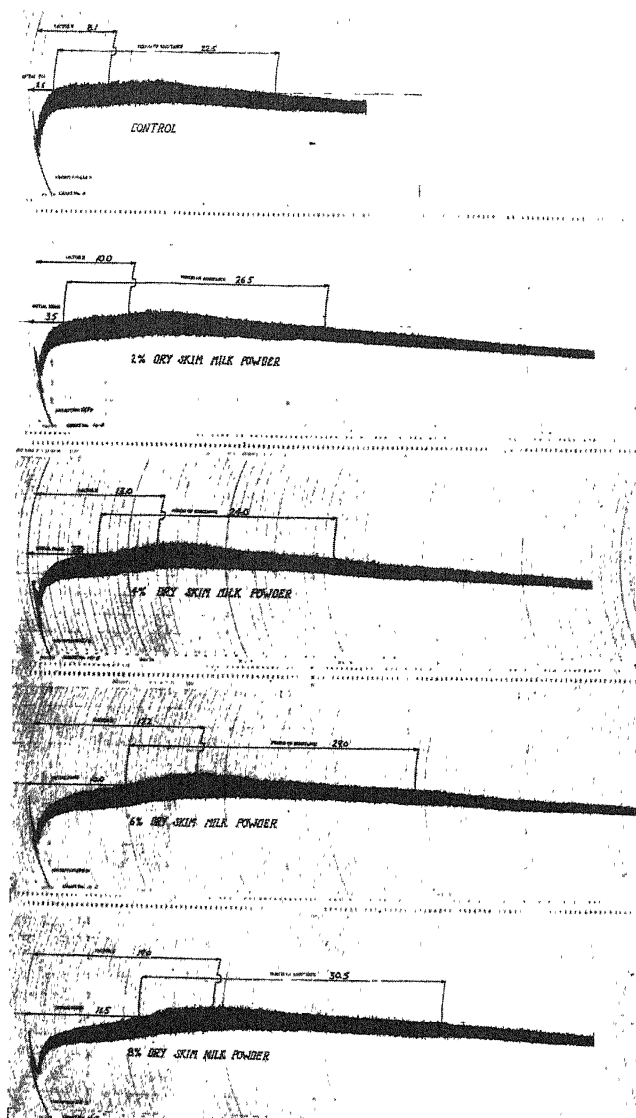


Fig 17. Effect of varying percentages of dry skim-milk powder.

period of resistance of 22.5 in the control. On the other hand, similar quantities of dry whole-milk powder had a much less pronounced effect, as represented by Table XVII and Figure 18. The absorption level

TABLE XVII
EFFECT OF VARYING PERCENTAGES OF DRY WHOLE-MILK POWDER

Dry whole-milk powder	Absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	min	min	
Control	65.8	2.5	22.5	8.1
2%	64.5	2.5	26.5	9.1
4%	64.8	3.5	27.5	10.5
6%	65.5	6.0	25.0	12.2
8%	66.5	8.0	24.5	14.0
EFFECT OF WHOLE LIQUID MILK				
100%	76.5	7.5	9.0	9.7

increased only from 64.5 to 66.5, while the initial phase increased to 8.0 and factor X to 14.0.

For comparative purposes, additional measurements were taken with fresh whole liquid milk in the control flour in place of the water. The absorption obtained with 100% liquid milk was 76.5 as compared with 65.8 for the control, while the initial phase increased to 7.5 in comparison with 2.5 for the control, the period of resistance dropped to 9.0 in comparison with 22.5 for the control; and the factor X increased to 9.7.

The combined effect of certain of these ingredients is clearly shown in Table XVIII and Figure 19. The two curves represented in this

TABLE XVIII
STATEMENT OF LEAN AND RICH FORMULAS

Lean commercial	Dough formulas	Rich commercial
2%	Yeast	2%
1 1/4%	Bromate-type dough conditioner	1 1/4%
1 1/2%	20°L malt	1 1/2%
2%	Salt	2%
3%	Cane sugar	6%
2%	Hydrogenated cottonseed oil	5%
2%	Dry skim milk	6%

classification cover doughs made from a typical commercial lean formula and a typical commercial rich formula. The formulas employed for these two doughs are tabulated in Table XVIII.

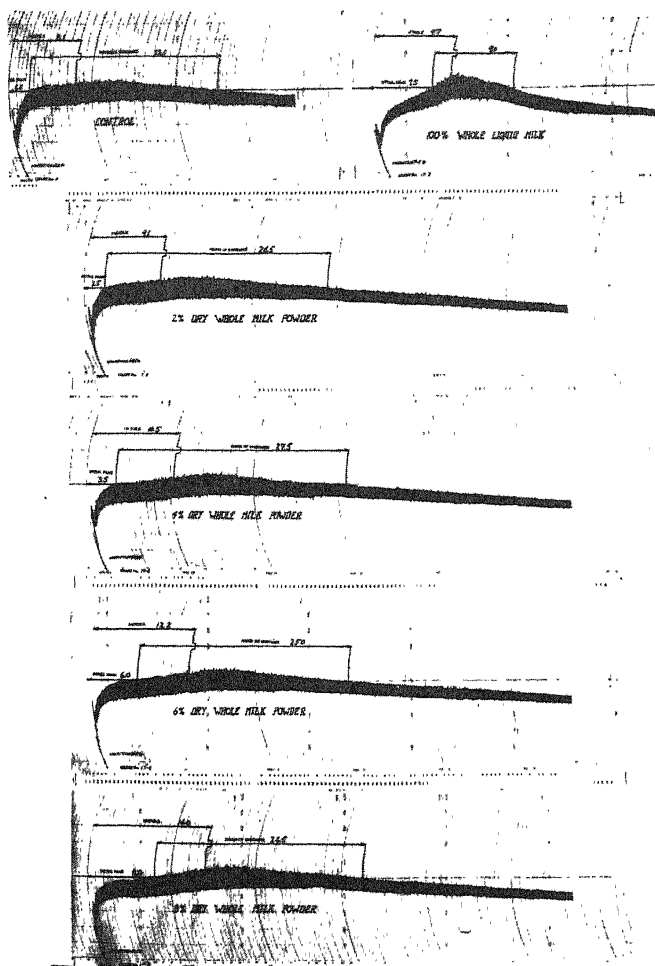


Fig 18. Effect of varying percentages of dry whole-milk powder.

In comparing the control dough with these two commercial types of bread-dough formulas (Table XIX), it will be observed that the initial phase has been most drastically affected, increasing from 2.5 for the control to 9.0 for the lean formula, and to 17.5 for the rich formula. Factor X has likewise increased from 8.1 for the control to 17.0 for the lean formula and 23.5 for the rich formula.

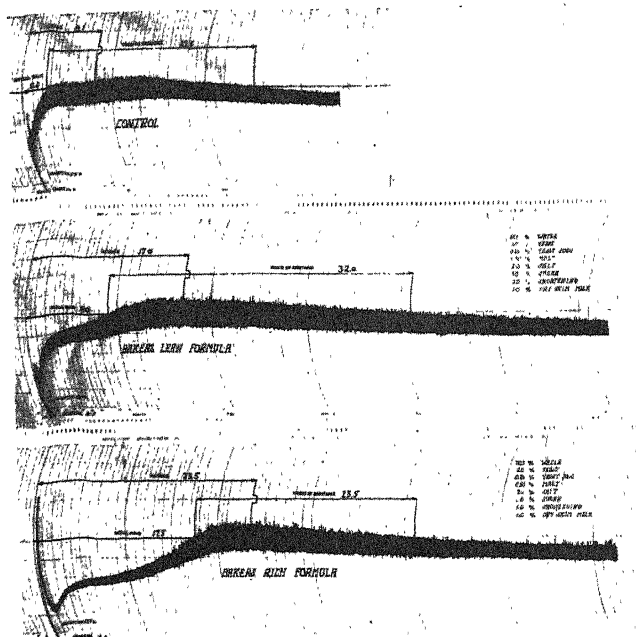


Fig 19. Effect of lean and rich commercial bread formulas

TABLE XIX
EFFECT OF LEAN AND RICH FORMULAS

	Percent absorption, 13.5% mb	Initial phase	Period of resistance	Factor X
	%	min	min	
EFFECT OF COMMERCIAL LEAN FORMULA				
Control	65.8	2.5	22.5	8.1
	64.7	9.0	32.0	17.0
EFFECT OF COMMERCIAL RICH FORMULA				
	69.9	17.5	23.5	23.5

Summary

Brabender farinograph curve characteristics are influenced in differing degrees through the addition of varying amounts of materials commonly employed in the commercial baking process. The effects obtained at a maintained temperature of 86°F with sodium chloride, compressed yeast, cane sugar, corn sugar, 20°L commercial malt syrup, 60°L commercial malt syrup, hydrogenated cottonseed oil, dairy butter,

leaf lard, bromate-type dough conditioner, peroxide-type dough conditioner, iodate- and phosphate-type dough conditioners, potassium bromate, dry skim-milk powder, dry whole-milk powder, and whole liquid milk, respectively, are reported.

In addition, the effects of varying temperatures and of differing dough consistencies are shown.

A proposed new system of farinograph curve measurement is suggested.

Acknowledgments

Acknowledgment is made to Roland Smith and Charles Schober for their manipulation of the Brabender farinograph.

Literature Cited

Brabender, C. W., and Hartkopf, Arthur

1938 Personal communications.

Skovholt, O., and Bailey, C. H.

1932 The effect of temperature and of the inclusion of dry skimmed milk upon the properties of doughs as measured with the farinograph. *Cereal Chem.* 12: 523-530.

TESTS WITH THE BERLINER-SCHMIDT COLORIMETRIC METHOD FOR THE RAPID DETERMINATION OF SUGARS IN WHEAT FLOUR

EDWIN ZIEGLER

Buhler Bros., Uzwil, Switzerland

(Received for publication February 2, 1942)

The object of the present paper is to describe a rapid method for determining diastatic activity which has proved very useful in mill control work in Europe. Numerous methods have been proposed for determining the "maltose figure," which is frequently used to control the work of the flour mill. Methods such as those of Rumsey, of Blish and Sandstedt (1933), and of Hildebrand and McClellan (1938) need a fair amount of skill besides numerous chemical solutions. The Kent-Jones (1929) method has been widely used during the last 15 years, but although simpler than the above-mentioned methods, the titration with methylene blue is somewhat difficult. There is a need for a rapid and very simple method for everyday work.

Colorimetric methods for the determination of sugar have involved the use of α -naphthol or picric acid. The latter was used by Blish, Sandstedt, and Platenius (1929) and by Kent-Jones and Saxby (1929). A photolorimetric method was proposed by Forsee (1938) which requires only one standard solution and is said to be rapid and accurate.

Recently a colorimetric method for determining sucrose using acid sodium selenite has been described by Mohs and Tornow (1940).

The present paper will describe experiments with the Berliner and Schmidt (1933) colorimetric method, which can also be used for the determination of pre-existing sugars (Berliner, 1939). The Blish and Sandstedt (1933) method with the Sandstedt (1937) modification was used for comparison. This method has been subject to some criticism and induced Hildebrand and McClellan (1938) to try a modification using ceric sulfate, which did not seem to give more reliable results. Popoff (1939) also proposed a modification. Klemen (1938) was not satisfied with the results obtained with the Blish and Sandstedt method, which gave results in disagreement with those obtained by some other methods.

Using the Blish-Sandstedt method, Davis (1937) only recovered 88.3% to 93.1% of the theoretical value with pure maltose; Bottomley (1938) obtained 84.0% to 94.1%. However, with the Kent-Jones method the latter recovered 98.8%-100.9% maltose. Blish and Sandstedt (1933) state that the relationship between milligrams of ferricyanide and maltose hydrate is linear; according to Redfern and Johnston (1938) this is true up to 84 mg of maltose.

Method

The Berliner and Schmidt (1933) method is based on the principle that certain sugars caramelize on being boiled in alkali. At first the method was standardized against the method of Berliner and Rüter (1928), not against pure maltose. The figures published in Ziegler's (1940) paper on the Berliner method, although comparative, are not identical with those of the present paper.

The method is now carried out as follows: Digest 10 g of flour with 50 ml. of water for 60 minutes at 27°C with continuous shaking. At the end of this time filter (e.g. through a Schleicher and Schüll filter paper, No. 588), pouring back the first few drops. After exactly 20 minutes of filtration transfer 15 ml of the solution to a test tube and add 5 ml of approximately normal sodium hydroxide (40 g per liter). Place the test tube in boiling water for exactly 5 minutes. During this time the yellow-colored solution becomes brown, the depth of shade depending on the amount of sugar present. Then cool the test tube, allowing the protein precipitate to settle for 30 to 60 seconds, and measure the depth of color in a colorimeter or compare with standard colors. The color is fairly stable.

Schmidt (1937) stated that protein does not affect the reaction. For comparison he made a series of standard color solutions with bichromate. He states (without giving proof) that the yellow color

given by the water-soluble indicator dyestuff in flour in the presence of alkali is not destroyed by the five minutes of boiling. Simpson (1935) has shown this color to be due to a flavone. We found the greenish tinge in flour extracts disturbing when using the standard colors, which only contain the yellow-brown shade resulting from the caramelization. Berliner¹ has found that this greenish color is due to the presence of sulfur and heavy metals.

Schmidt (1938) has shown that the concentration of alkali can be chosen within wide limits. With concentrations between 0.2*N* and 2*N* no differences were found. He also mentions that the following sugars were caramelized: maltose, glucose, levulose, mannose, arabinose, lactose, galactose, and glycerose. On the other hand the following substances were not caramelized: sucrose, raffinose, starch, dextrin, mannite, and sorbite. He mentions that by caramelization the reducing power of maltose was only partly destroyed. For measuring the maltose figure he advocates the Schmidt and Kühn sugar colorimeter, which embodies a series of colored-glass standards. Berliner and Kranz (1937) advocate the use of a photoelectric colorimeter. The Berliner-Schmidt method is now used in Germany as an official method.

Experimental

For the digestion a rotating thermostat was used with stoppered wide-necked test tubes. Six to 10 test tubes with the filtrate plus alkali were put into a round test-tube holder which was first transferred to the boiling water, then to the table, and finally into the cold water for cooling. The measurement was made in a Buhler photoelectric colorimeter, using a blue filter. This instrument is designed to equalize current disturbances. In the following figures and tables 100% absorption indicates complete darkness and 0% maximum transmittency. The standardization of the instrument was kindly done by Dr. Berliner in Darmstadt. The pure maltose used was Merck's maltose hydrate.

Figure 1 shows the relationship found between maltose and light absorption. Less than 0.25% maltose could not be measured. The usual values in wheat-flour work lie between 1.3% and 3%.

The relationship between Berliner's curve and pure maltose is shown in Figure 2. Since a 20% suspension is used, 15 ml of filtrate contains the maltose formed by 3 g of flour. Thus 30 mg of pure maltose equals 1% maltose. Solutions were made up with pure maltose hydrate in 15 ml of water and treated with 5 ml of alkali as stated above. In Figure 2, curve A is the theoretical curve, whereas curve B is the one actually found. Between 40 mg and 105 mg (1.3%–3.4%), the recovery was 100%. Above and below these limits—i.e. outside

¹ Private communication.

the range of practical values in mill control work—the Berliner figures were somewhat low. At the 4% level 98.5% was recovered, at the 5% level 96%. Curve C will be referred to later on.

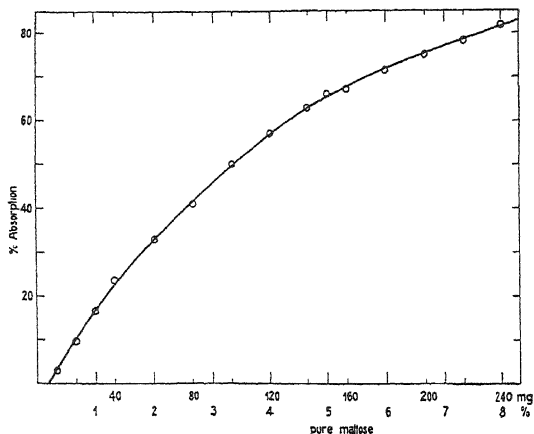


Fig. 1 Light absorption in a photoelectric colorimeter by different amounts of caramelized maltose.

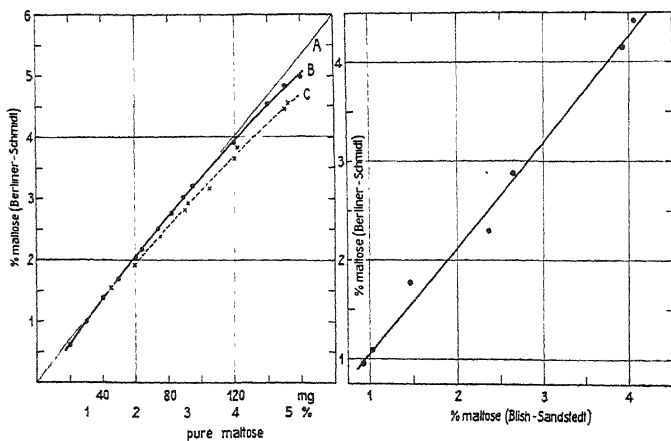


Fig. 2. Relationship between the maltose figure (Berliner-Schmidt method) and pure maltose. A = theoretical curve. B = actual curve. C = maltose recovered after the addition of increasing increments of maltose to flour. Fig. 3 (Right). Comparison of the Blish and Berliner methods.

The Blish-Sandstedt and Berliner-Schmidt methods were compared with each other (Table I). For seven flour samples the difference between extreme values on five different days was 8, 8, 6, 11, 12, 14,

TABLE I
COMPARISON OF TWO METHODS FOR DETERMINING MALTOSE
IN FLOUR EXTRACTS

(Single results obtained on five different days)

Flour sample	Blish-Sandstedt method— mg maltose per 10 g flour						Berliner-Schmidt method— % maltose					
	1	2	3	4	5	Mean	1	2	3	4	5	Mean
1	94	90	94	94	94	93	0.92	1.00	0.95	0.97	0.97	0.96
2	104	94	100	100	100	100	1.03	1.11	1.05	1.11	1.11	1.08
3	150	140	150	150	145	147	1.75	1.80	1.74	1.80	1.80	1.78
4	234	227	237	246	237	236	2.30	2.30	2.23	2.34	2.30	2.30
5	266	256	266	266	264	264	2.85	2.93	2.81	2.89	2.93	2.88
6	403	387	396	392	390	394	4.19	4.24	4.10	4.10	4.10	4.15
7	406	400	408	416	406	407	4.50	4.50	4.34	4.39	4.45	4.42

and 16 mg per 10 g of flour for the Blish method and 4, 10, 10, 19, 10, 16, and 16 mg for the Berliner method. The mean values of the seven samples are shown in Figure 3, according to which the Blish values are 94% of the Berliner values, a figure which corresponds with those of Davis (1937) and Bottomley (1938), referred to above.

Table II shows the effect, on the absorption of the water-soluble indicator dyestuff, of the addition of alkali to two flours with widely

TABLE II
COLOR OF THE FILTRATE ON ADDITION OF ALKALI BEFORE AND AFTER
BOILING BY THE BERLINER AND SCHMIDT METHOD

	Flour A, 0.49% ash	Flour B, 1.17% ash
	ABSORPTION, %	
Filtrate alone	2.7	5.5
" + NaOH (before heating)	4.0	9.5
" + NaOH (after heating)	28.0	25.5
	MALTOSE, %	
Filtrate + NaOH (after heating)	1.67	1.50

varying ash content but of similar maltose figure. The darkening of color due to caramelization and possibly other transformations is almost twice as great for the low-ash flour as compared with the other sample. This point needs further investigation.

Recovery of Added Maltose

Table III shows that on addition of pure maltose to flour before autolysis, both sugar methods gave only a partial recovery, which was of the same magnitude for both methods, if we take into account that the Blish values are roughly 94% of the Berliner figures. Figure 2,

TABLE III
DETERMINATION OF MALTOSE CONTENT OF A FLOUR WITH
ADDED PURE MALTOSE

Maltose added to flour, %	Bligh-Sandstedt method (flour A)			Berliner-Schmidt method (flour B)		
	Found	Calculated	Recovery	Found	Calculated	Recovery
	<i>mg maltose per 10 g flour</i>			<i>% maltose</i>		
0	175	—	—	1.57	—	—
1	260	275	94	—	—	—
1.5	—	—	—	2.93	3.07	96
2.0	348	375	93	—	—	—
2.5	—	—	—	3.82	4.07	94
3.0	422	475	89	—	—	—
3.5	—	—	—	4.56	5.07	90
4.0	488	575	85	—	—	—

curve C, gives further values for the Berliner method. A few tests were carried out to see where the loss might originate (Table IV). Addition to the flour filtrate gave a 100% recovery (Table IV, Nos. 1-4). Tests on various samples of flour on different days (Nos. 5 and

TABLE IV
RECOVERY OF ADDED MALTOSE
(Maltose determined on 15 ml of filtrate by the Berliner method—
recovery given in parentheses)

	Maltose		
	%	%	%
1. 15 ml flour filtrate alone	1.50	—	—
2. 15 ml water + 60 mg maltose	1.94	—	—
3. 15 ml flour filtrate + 60 mg maltose	3.43 (100%)	—	—
4. Calculated value from 1 and 2	3.44	—	—
5. Flour-water suspension (20%) alone	1.50	1.50	1.70
Maltose added	(198 mg)	(350 mg)	(270 mg)
6. Maltose added before autolysis	3.34 (93%)	4.77 (95%)	4.34 (98%)
7. Maltose added after 1 hr autolysis	—	—	—
before filtering	3.39	4.77	4.24
8. 81 mg maltose to 15 ml filtrate	—	—	4.24
9. Maltose solution alone	1.98	2.02	2.59
10. + 7 g dried wheat starch	1.94	2.00	2.59
11. + wet gluten from 10 g of flour	1.83 (93.5%)	1.92 (95%)	2.26 (87%)
Average recovery: 92%			

6) gave results similar to those of Table III. On the average the difference was the same whether the maltose was added before or after autolysis (Nos. 6 and 7), but before filtering. To ascertain whether the starch or the gluten was responsible for the loss through adsorption, these two products were taken from 10 g of flour and shaken for one

hour with a maltose solution. The sugar was then determined in 15 ml of filtrate. Whereas the starch had no definite effect, the wet gluten retained about 8% maltose.

Since this method can be carried out even in the simplest of laboratories and needs no great skill it would seem appropriate that collaborative testing of the method be carried out.

Conclusion

The Berliner and Schmidt colorimetric sugar method is most useful for daily control work, because of its simplicity, rapidity, and reliability.

Literature Cited

- Berliner, E.
1939 Einige Schnellanalysen für das Mühlenbetriebslaboratorium. *Mühlenlab.* 9: 57-64.
— and Ruter, R.
1928 Titrimetrische und polarimetrische Zuckerbestimmung. *Z. ges. Mühlenw.* 5: 116-120.
— and Schmidt, E.
1933 Über Biuretreaktion, Trommersche Zuckerprobe und eine neue, einfache kolorimetrische Zuckerbestimmung. *Mühlenlab.* 3: 187-192.
Blish, M. J., and Sandstedt, R. M.
1933 An improved method for the estimation of flour diastatic value. *Cereal Chem.* 10: 189-202.
— — and Platenius, H.
1929 Correlation between diastatic power of flour and crust color in the test loaf, and its significance. *Cereal Chem.* 6: 121-127.
Bottomley, R. A.
1938 The relationship between diastatic activity and "gassing power" of experimentally milled flours from some Australian wheats. *Cereal Chem.* 15: 509-520.
Davis, C. F.
1937 A collaborative study of the Blish-Sandstedt, Schoorl, and Bertrand methods for determining reducing sugars in flour diastatic activity measurements. *Cereal Chem.* 14: 81-85.
Forsee, W. T., Jr.
1938 Determination of sugars in plant materials. A photolorimetric method. *Ind. Eng. Chem. (Anal. Ed.)* 10: 411-412.
Hildebrand, F. C., and McClellan, B. A.
1938 An improved method of sugar determination in diastatic activity measurements. *Cereal Chem.* 15: 107-113.
Kent-Jones, D. W., and Saxby, J.
1929 Die diastatische Kraft der Mehle. *Z. ges. Getreidew.* 16: 171-177.
Klemen, R.
1938 Über die Verwendbarkeit einzelner Eiweißfallungsmittel bei der Zuckerbestimmung in Getreideautolysaten. *Biochem. Z.* 299: 63-68.
Mohs, K., and Tornow, Elisabeth
1940 Keimenergie und Selenreduktion. *Z. ges. Getreidew.* 27: 17-22.
Popoff, I. D.
1939 Über eine Methode zur Bestimmung der diastatischen Kraft der Getreidemehle und einige Faktoren, welche die diastatische Wirkung beeinflussen. *Biochem. Z.* 302: 177-186.
Redfern, S., and Johnston, W. R.
1938 The effect of the substrate on diastatic activity. *Cereal Chem.* 15: 327-341.
Sandstedt, R. M.
1937 The adaptation of the ferricyanide maltose method to high diastatic flours. *Cereal Chem.* 14: 603-604.

- Schmidt, E.
1937 Die Biuretreaktion als mehchemische Analyse nebst einer schnell ausführbaren Maltosebestimmung. Thesis, Berlin.
1938 Eine kolorimetrische Zuckerbestimmung. *Mühlenlab.* 8: 121-132.
- Simpson, A. G.
1935 A simple method for determining the "yellowness" and grade of wheat flours. *Cereal Chem.* 12: 569-574.
- Ziegler, E.
1940 The effect on diastatic activity of surface, pressure, differential and temperature of the reduction rolls in milling. *Cereal Chem.* 17: 668-679.

THE ACTION OF HYDROGEN PEROXIDE ON WHEAT FLOUR WHEN USED IN COMBINATION WITH REDUCING AGENTS

E. ELION

Baltimore, Maryland

(Read at the Annual Meeting, May 1942)

Hydrogen peroxide has been proposed as a dough-leavening agent, either without or in combination with yeast. The production of oxygen gas through the action of flour catalase on the hydrogen peroxide serves as a leavening agent in such methods.

Although hydrogen peroxide is widely used as a bleaching agent (Makow, 1932), it is not commercially used in flour bleaching (Roell, 1934). While Neumann (1929) reports that hydrogen peroxide has a flour-bleaching action, Bailey (1925) states that hydrogen peroxide solution shaken with a benzene extract of flour did not bleach the flour pigments dissolved in the latter.

It has long been known that flour contains the enzyme catalase, which decomposes hydrogen peroxide into water and oxygen. Gelissen (1924) and Van der Lee (1932) show that very small amounts of certain oxidizing agents, such as chlorine (Gelissen) and chlorates, perchlorates, bromates, iodates, periodates, and persulfates (Van der Lee) inhibit the action of catalase on hydrogen peroxide.

Blish and Bode (1935), in a study of catalase activity in wheat flour, report that among flours of comparable grade, but of widely diversified origin, catalase activity varies widely and systematically with the regional or climatic origin of the samples.

The purpose of the present investigation has been to determine whether reducing compounds, such as ascorbic acid, can serve as a means of inhibiting the catalase activity, so that all flours could be brought to the same catalase activity.

Experimental

For purposes of this investigation the absolute value of the catalase activity of the flour used was immaterial, and for most of the experi-

ments an unbleached high-protein straight-grade Texas flour was selected.

Experiments were made according to the method described by Van der Lee (1932): 10 g of wheat flour was thoroughly mixed with 50 ml of water, whereupon 20 ml of an approximately 1.5% solution of hydrogen peroxide was added. In another experiment 20 mg of reducing agent was first dissolved in the 50 ml of water. Both samples were allowed to stand for an hour at room temperature, then filtered rapidly and in aliquot parts of the filtrate the remaining hydrogen peroxide was determined. In a blank experiment the mixture was filtered immediately upon mixing, without standing for an hour, and analyzed. Since in the blank experiment measurable decomposition of hydrogen peroxide occurred during the filtration of the sample, *l*-ascorbic acid was added to retard such decomposition, in order to obtain a more reliable value for the zero time. Some results obtained are given in Table I.

TABLE I
DECOMPOSITION OF HYDROGEN PEROXIDE BY WHEAT FLOUR CATALASE

Duration of catalase action	Material added	Remaining hydrogen peroxide
hrs		%
0	—	100
1	—	38.9
1	<i>l</i> -Ascorbic acid	76.9
1	Pyrogalllic acid	69.4
1	Gallic acid	77.8
1	Pyrocatechin	77.8
1	Carminic acid	88.9
1	Maleic acid	100
1	Formaldehyde	87.5
1	Sodium hypophosphite	62.9
1	Potassium metabisulfite	85.2
1	Sodium thiosulfate	48.1
1	Ferrous sulfate	76.9
1	Ferrous chloride	81.5
1	Stannous chloride	91.7

Under the conditions of these experiments, when no chemical was added, only 38.9% of the original amount of hydrogen peroxide remained undecomposed after one hour's action of the flour catalase. All the materials used caused considerable retardation of the hydrogen peroxide decomposition.

Similar results have been obtained with urea peroxide, $\text{CO}(\text{NH}_2)_2 \cdot \text{H}_2\text{O}_2$, instead of hydrogen peroxide, which may be due to the presence of hydrogen peroxide in the urea peroxide solution (Table II).

In the preceding experiments the quantity of reducing agent amounted to 0.2% of the flour weight. It was then appropriate to

TABLE II
DECOMPOSITION OF UREA PEROXIDE BY WHEAT FLOUR CATALASE

Duration of catalase action	Material added	Remaining urea peroxide
<i>h s</i>		%
0	—	100
1	—	39.0
1	<i>l</i> -Ascorbic acid	78.9
1	Gallic acid	87.1

investigate whether smaller amounts of these chemicals have a similar retarding action on the decomposition of hydrogen peroxide by flour catalase. The experiments reported in Table III have been made with

TABLE III
DECOMPOSITION OF HYDROGEN PEROXIDE BY WHEAT FLOUR CATALASE
(With half the concentration of hydrogen peroxide shown in Table I)

Duration of catalase action	<i>l</i> -Ascorbic acid in the flour	Remaining hydrogen peroxide
<i>h s</i>	%	%
0	0	100
1	0	20.3
1	0.2	80.0
1	0.1	70.7
1	0.05	56.3
1	0.01	38.6
1	0.005	32.7
1	0.001	28.2
1	0.0005	19.7

l-ascorbic acid as reducing agent and only half the concentration of hydrogen peroxide used in the experiments of Table I.

With decreasing amounts of reducing agent the inhibiting action on the catalase also decreased, thus providing a means to adjust the catalase activity of different flours to the same level.

Summary

Wheat flour contains catalase which decomposes hydrogen peroxide and thus may prevent bleaching by added hydrogen peroxide. The results show that several reducing agents, when present in small amounts during the action of catalase upon hydrogen peroxide, considerably retard the catalase decomposition of hydrogen peroxide.

Literature Cited

- Bailey, C. H.
1925 The chemistry of wheat flour. The Chemical Catalog Company, Inc., New York, N. Y.
Blish, M. J., and Bode, C. E.
1935 Catalase activity in wheat flour. Cereal Chem. 12: 133-142.

- Gelissen, H. C. J. H.
1924 Process for bleaching and improving flour and milling products. U. S. Patent 1,483,546.
- Makow, V.
1932 F. Ullmann, Enzyklopädie der technischen Chemie, Vol. X. Urban & Schwarzenberg, Berlin, Wien.
- Neumann, M. P.
1929 Brotgetreide und Brot. Paul Parey, Berlin
- Roell, L.
1934 Die Backmittel. W. Engelmann, Leipzig.
- Van der Lee, G.
1932 Process for enhancing the action of percompounds which are decomposed by catalases upon animal or vegetable products. U. S. Patent 1,866,412.

A METHOD OF DETERMINING DEGREE OF COOKING IN CEREAL PRODUCTS

G. H. BENHAM

Department of Chemistry, Macdonald College, St. Anne de Bellevue, Quebec, Canada

(Received for publication April 20, 1942)

The concept of "alkali lability" as applied to starch was developed by Taylor and Salzmänn (1933), who recognized two distinct fractions, the alkali-labile and the alkali-stable. The former was quantitatively defined in terms of the amount of alkali taken up by the starch during treatment with hot aqueous alkali. Their practical method was based on the neutralization of the remaining alkali after alkali digestion under a standard set of conditions, followed by iodometric determination of the reducing substances. This method was found to be a tedious one, and gave place to the simplified idea of "alkali numbers" described by Schoch and Jensen (1940). To quote their own words: "Raw starch slowly decomposes in hot aqueous alkali to give simple acidic substances, principally formic, acetic and lactic acids, as well as pyruvic aldehyde. These acidic substances are determined by means of a back-titration technique similar to that used for the saponification of a fat."

Their definition is: Alkali number = ml 0.1*N* NaOH/g starch/1 hour's digestion at 100°C.

Although such a number is an empirical quantity only, and furthermore does not give a direct measurement of aldehyde content, it is of value in following the hydrolytic state of a starch or starchy material. The heterogeneity of raw cornstarch is well shown by Schoch and Jensen, while differences of source material, preparation, and pretreatment for manufacturing purposes are reflected in a wide range of alkali numbers, *viz.* from 6 to 66. The interpretation of these figures on the basis of starch "degradation" awaits further fundamental research, but the practical possibilities of the alkali-number technique are considerable.

Accordingly, it was decided to apply this concept to the assessment of cooking, to see whether a degree of cooking can be estimated by relating it to the alkali number. As far as the writer is aware, there is no ready-to-hand measurement of the degree of cooking, since the very nature of the cooking process is little understood. That the cooking of starch is associated with its gelatinization is obvious, but the extent of chemical, as distinct from physical, change in such a gelatinization process needs to be known. Cooking is not necessarily associated with "dextrinization," which is a loose term to define many unknowns.

Workers in the field have used gelatinization as a criterion (Cunningham, 1931; Hughes, Green, and Campbell, 1938), but this is hardly satisfactory. There is a need, as was stressed 14 years ago by Miner (1928), for a quick means of computing the degree of cooking of cereal products. This paper describes an attempt to develop a procedure that would facilitate control work in all cases where partial or complete cooking of a cereal product is desired during its manufacture.

There are three sections: (1) a critical repetition of Schoch and Jensen's procedure, (2) an investigation into the alkali numbers of various starches, flours, and cereal products as received from mill or manufacturer, and (3) a working experiment on cooking under various conditions, following alkali numbers at each stage.

The Schoch and Jensen Method

The procedure as described by Schoch and Jensen was carried out in detail. Certain modifications were tried, with the results shown in Table I. Each figure represents numerous determinations, all carried

TABLE I
MODIFICATIONS OF THE SCHOCH AND JENSEN PROCEDURE WITH WHEAT STARCH

Conditions	Alkali number	Percent of true value
1. Method as described by Schoch and Jensen	8.3	100
2. Water bath just off the boil	5.0	61
3. Ten ml alkali used instead of 25 ml	3.9	47
4. Larger quantities of starch than the 500 mg specified	5.7	69
5. Volume of total liquid halved	6.7	81
6. Method as described by Schoch and Jensen, but titrate hot	8.3	100
7. No blank calculation	9.1	110

out on one wheat starch, with one variable altered at a time. Of the precautions which these workers observed it was found that only one seemed unnecessary; *viz.*, it did not make any difference whether the bottles were cooled before the titration, provided it was carried out at once.

The following conclusions were drawn from the results shown in Table I (numbers refer to conditions):

2. It is necessary to space the determinations at least five minutes apart, to prevent the water bath from coming off the boil when the bottles are introduced. Accordingly ten determinations may be run in a total working time of two hours.

3. It is necessary to use 25 ml of alkali rather than 10 ml for digestion, even though this necessitates a large quantity of acid for back-titration.

4. In order to decrease percentage error in burette readings, a larger quantity of starch was used. However, this gave low results, so that very accurate burette reading, if possible to 0.02 ml, becomes necessary. It was calculated that with this technique the accuracy of manipulation is such that alkali numbers may be determined to ± 0.25 unit. By doing each determination in triplicate, an accuracy of 0.1 unit is claimed.

5. Halving the total volume by cutting the water added, but using the same amount of alkali, gives low results also. However, with practice it becomes quite easy to detect a true end point with the larger volume, although in this connection a personal preference was shown for phenolphthalein rather than thymol blue.

6. If back-titration is done at once, the end point is maintained long enough for a reading, so that it becomes unnecessary to spend time cooling the bottles.

7. The blank titer is subtracted from the final titer. In cases where the alkali number is low, the blank is particularly important; for instance a typical titer of 3 ml will have a blank titer of 0.3 ml, which if not accounted for gives a value 10% too high.

Following this survey, which accurately substantiates Schoch and Jensen's precautions, the procedure adopted was standardized as follows:

Starch or starchy material (always sifted or ground, as the case may demand, to pass a 60-mesh sieve) is digested for one hour exactly at 100°C with 25 ml of about 0.4*N* NaOH. Back titration with accurately standardized H₂SO₄ (about 0.2*N*) is done immediately the 60 minutes are up, using phenolphthalein as indicator. In each case triplicate determinations are made, together with a blank in which distilled water is substituted for the alkali.

The alkali number is then represented as follows:

$$\frac{(\text{ml acid for back-titration}) - (\text{ml acid corresponding to blank titration})}{\times \text{normality of acid} \times 10}$$

g of starting material on dry basis.

Investigation of Various Cereal Products

Using the above technique, triplicate determinations were made on starches, flours, and certain cereal products as obtained from the manufacturer. Table II is arranged in ascending order of alkali numbers.

TABLE II
ALKALI NUMBERS OF VARIOUS CEREAL PRODUCTS

Material	State of material	Alkali number
Corn starch	Raw	7.4
Wheat starch	Raw	8.3
Potato flour	Raw	8.6
Farina	Raw	11.2
Rye flour	Raw	12.7
Buckwheat flour	Raw	14.4
Corn flour	Raw	14.8
Wheat flour (low grade)	Raw	15.3
Rolled oats	Raw	15.7
Oat flour	Raw	16.5
Barley flour	Raw	17.0
Farinaceous breakfast food (1)	Precooked	19.0
Bread (crumb)	Cooked	20.1
Bread (light crust)	Cooked, lightly toasted	20.7
Cold breakfast cereal (1)	Cooked, toasted	21.0
" " " (2)	" "	21.2
" " " (3)	" "	21.6
"Pablum"	Precooked	24.0
Cold breakfast cereal (4)	Cooked, toasted	25.2
Farinaceous breakfast food (2)	Precooked	26.6
Bread—dark crust	Cooked and toasted	27.1
Cold breakfast cereal (5)	Cooked and toasted	27.9
Cold breakfast cereal (6)		29.7
Soluble starch		65.0
Maltose (theoretical value)		84.5
Glucose (theoretical value)		85.2

From the data in Table II the following conclusions can be drawn: (1) Raw starches have the lowest values, and the corresponding flours are higher. (2) All true raw materials in the table have values of 17 or under. (3) All cooked, pretreated, flaked, and toasted products have values of 19 or over. (4) Evidently a certain hydrolysis of material does take place during cooking and especially during the toasting process. (5) The data indicate an increased alkali number of the starch in cooked material, but it must be pointed out that any changes in the hydrolytic state of the protein also influence the figures.

It must be emphasized here that care should be taken over toasted cereals, which contain added material. Whereas the table shows that cooked cereals in general have much higher values than raw flours, these figures are not comparable, owing to the great differences in sources, material, and treatment.

The only real test for the adaptation of this method to assess degree of cooking is to take a given material and cook it in various ways and to various extents. This has been done using farina and a low-grade wheat flour. These experiments are described in the following section.

Alkali Numbers at Various Stages of Cooking

The variables associated with cooking are as follows: (1) fineness of material, (2) percentage of water in material to be cooked, which is in other words the consistency of the material prior to cooking, (3) time, and (4) temperature and pressure.

These have been investigated in turn, and the alkali numbers of the product after the given treatment have been determined in triplicate according to the technique just described. In each table the percentage of increase due to the treatment is given in the last column.

Fineness of material: It is well known that the coarser the granularity of a material, the longer it takes to cook. There is a marked difference in the alkali numbers of a granular product and of the same product ground to flour fineness when submitted to identical cooking procedures.

TABLE III
FINENESS OF MATERIAL AND ALKALI NUMBER

	Alkali number	Percent increase
Farina—raw material, unground, no cooking	11.2	
Farina—raw material unground, gelatinized until thick in 5 ml hot water	16.4	47
Farina—raw material ground to pass 60-mesh sieve, gelatinized until thick in 5 ml hot water	21.2	90

Table III shows that it is necessary before any cooking treatments to grind granular material to a uniform flour fineness to eliminate this variable. This has been done throughout all alkali number estimations.

Percentage of water: The flour-water ratio for optimum cooking was investigated and the results are shown in Tables IV and V. Cooking consisted of simmering gently, with stirring, in small beakers. It is advisable to use a low gas flame and two gauzes.

A lowering of the amount of water as shown in Tables IV and V enhances the cooking of a raw starchy material, the optimum ratio given for a cereal like oatmeal being 1 to 6 or 7. The alkali number changes markedly with the flour:water ratio; the most favorable conditions for farina, 1:6, gave over 100% increase, while 1:7 ratio in the low-grade flour gave 50% increase.

TABLE IV
FLOUR-WATER RATIO AS RELATED TO ALKALI NUMBER—FARINA

	Alkali number	Percent increase
Raw farina	11.2	—
Cooked 2 minutes; flour : water 1 : 20	16.5	47
" " " " " " 1 : 10	16.4	46
" " " " " " 1 : 6	16.6	48
Cooked 5 minutes; flour : water 1 : 10	19.0	70
" " " " " " 1 : 6	22.9	105

TABLE V
FLOUR-WATER RATIO AS RELATED TO ALKALI NUMBER—LOW-GRADE FLOUR

	Alkali number	Percent increase
Raw flour	15.3	—
Cooked 5 minutes; flour : water 1 : 10	17.1	12
" " " " " " 1 : 9	19.3	26
" " " " " " 1 : 8	22.3	46
" " " " " " 1 : 7	23.0	50

Time: We know that there is a point at which a product is what we call "thoroughly cooked," so that further cooking is useless irrespective of time and temperature. At this point, as shown below, the alkali number also reaches a maximum, and it is this apparent correlation which is stressed in this paper.

TABLE VI
TIME OF COOKING AND ALKALI NUMBER—LOW-GRADE FLOUR

	Alkali number	Percent increase
Raw material	15.3	—
Cooked in 1 : 8 ratio for 1 minute	16.6	8
" " " " " " 2 minutes	16.3	6
" " " " " " 3 "	16.5	8
" " " " " " 4 "	19.5	27
" " " " " " 5 "	22.3	46
" " " " " " 10 "	24.0	58
" " " " " " 15 "	23.2	52

Temperature and pressure: The temperature of cooking was raised from 100° to 125°C by carrying out the experiments in a pressure cooker at 17 pounds per square inch.

Neither increase in time nor increase in temperature and pressure (Table VII) raises the alkali number further than a certain critical value. It is evident that, within the experimental difficulties of standardizing a cooking procedure, a completion of cooking is directly associated with a leveling off of the alkali-number increase.

TABLE VII
TEMPERATURE AND PRESSURE—WITH LOW-GRADE FLOUR

	Alkali number	Percent increase
Raw material	15.3	—
1 : 8 ratio 5 minutes ordinary cooking, atmospheric pressure at 100°C	22.3	46
1 : 8 ratio 5 minutes pressure cooking, 17 lbs per sq in at 125°C	22.4	47

A further experiment was carried out to establish the alkali numbers after a low-grade flour was cooked at higher flour : water ratios. These ratios ranged from 1 : 3/5, a 60% absorption dough, through pastes, up to a 1 : 10 ratio, which is a thin porridge.

The various doughs and pastes were made up and submitted all at one time to 10 minutes of pressure cooking. The results are shown in Table VIII.

TABLE VIII
PRESSURE COOKING AND ALKALI NUMBER—LOW-GRADE FLOUR

	Alkali number	Percent increase
Raw material	15.3	—
Ratio 1 : 3/5 consistency of dough	15.5	2
" 1 : 7/10 " " "	18.2	19
" 1 : 3/4 " " "	18.9	23
" 1 : 2/3 " " thin dough	22.4	46
" 1 : 1 " " thick paste	24.3	59
" 1 : 1 1/2 " " paste	24.9	63
" 1 : 2 " " thin paste	22.7	48
" 1 : 7 " " thick porridge	23.0	50
" 1 : 8 " " porridge	22.4	46
" 1 : 9 " " thin porridge	19.3	26
" 1 : 10 " " thin porridge	17.1	12

The 1 : 3/5 dough appeared dry, rubbery, and yellow after this treatment. Increase of water to 1 : 4/5 ratio rendered a softer, whiter mass and there is a striking increase in the alkali number in this range. Increases in alkali number up to 60% are obtained with the pastes, which cook readily; remain white, and become rather fluid in the cooking.

TABLE IX
A PRECOOKED FARINACEOUS FOOD

	Time	Alkali number
As is	—	23.7
Cooked 1 : 8	2 minutes	23.7
Cooked 1 : 8	4 minutes	24.7
Cooked 1 : 8	5 minutes	24.0

On the other hand, when a "precooked" cereal food is submitted to cooking treatment, however drastic, there is no increase in alkali number; this is shown in Table IX.

Discussion

It has been long known that the addition of certain chemical substances helps the gelatinization of starches (Meusel, 1886); that the addition of dilute mineral or weak organic acids brings about starch hydrolysis (Kirchoff, 1811); and that enzymatic digestion of starch with malt, for instance, eventually causes complete hydrolysis to simple sugars (Guérin, 1835).

Cooking as referred to throughout this paper does not include any of these phenomena. The hydrolysis of starch to dextrins and finally to sugars normally takes place in the body and not during cooking. The alkali-number technique does not measure the same thing as the copper-number or iodometric techniques for starch breakdown products. It deals with the very early "depolymerization" of starch, which evidently takes place along with the rupture of the starch granule; this phenomenon may be likened to a type of loosening of the starch structure accompanied by an unknown but very slight amount of degradation.

For the theoretical aspects of starch depolymerization, reference is made to two important sets of papers recently published (Meyer, Brentano, and Bernfeld, 1940; Lampitt, Fuller, and Goldenberg, 1941). No attempt is made to theorize on the changes which we have followed in this paper. The effect of the presence of protein in raising the raw alkali number (*e.g.* cornstarch 7.4, corn flour 14.8) must be taken into account, with the probability that cooking affects the protein as well as the starch. But it is evident that the change consequent upon cooking any starchy material is a real one, and can be followed with a simple practical technique. Values are obtainable for any raw cereal product and for the extent of increase in cooking for the material under consideration. The more precise interpretation must await further work such as Dr. Meyer is undertaking, but at present this method serves as a useful guide in the cooking of cereal foods.

Summary

A method for assessing the degree of cooking of a starch or a starch cereal has been developed, using the principle of alkali lability.

Values for the "alkali number" of several products are given, ranging from 7.4 for raw corn starch to 30 for a toasted cereal.

Complete cooking of a starchy product raises the alkali number—by 60% in a low-grade flour and by 100% in a low-protein farina.

Cooking a precooked cereal has, however, no effect on the alkali number.

Literature Cited

- Cunningham, N. T.
1931 The cooking of cereal porridges. *Cereal Chem.* 8: 403.
- Guérin, V.
1835 Memoire concernant l'action de la diastase sur l'amidon de pomme de terre. *Compt. rend.* 1: 81.
- Hughes, O., Green, E., and Campbell, L.
1938 The effect of time and temperature on the percentage of gelatinization of wheat and corn starch and of cereals containing those starches. *Cereal Chem.* 15: 795.
- Kirchoff, G. S. C.
1811 *Tekhnologicheskii Zhurnal*, St. Petersburg 9: 3-26.
- Lampitt, G. H., Fuller, C. H. F., and Goldenberg, N.
1941 The fractionation of wheat starch. *J. Soc. Chem. Ind.* 60: 1-304.
- Meusel, E.
1886 Untersuchung uber die Quell Kraft der Rhodonate. *Jahrsberichte* 2099 (1886).
- Meyer, K. H., Brentano, W., and Bernfeld, P.
1940 The fractionation of starch. *Helv. Chim. Act.* 23: 845-884.
- Miner, C.
1928 Problems of the nonflour cereal chemist. *Cereal Chem.* 5: 70.
- Schoch, J. J., and Jensen, C. O.
1940 A simplified alkali lability determination for starch products. *Ind. Eng. Chem. (Anal. Ed.)* 12: 531.
- Taylor, T. C., and Salzmann, G. M.
1933 The action of aqueous alkali on starches, amyloses, and modified starches. *J. Am. Chem. Soc.* 55: 264.

PRECISION OF FLOUR MOISTURE RESULTS BETWEEN AND WITHIN LABORATORIES¹

W. F. GEDDES and C. A. ANKER

Division of Agricultural Biochemistry, Minnesota Agricultural Experiment Station,
University Farm, St. Paul, Minnesota

(Read at the Annual Meeting, May, 1942)

The importance of determining the reliability of various routine analytical procedures employed by the cereal chemist has been clearly recognized, as evidenced by the rather extensive list of experimental errors that have been compiled in the fourth edition of *Cereal Laboratory Methods*. Statistical analyses by Treloar and Harris (1928), Treloar (1932), and Davis and Wise (1933) of data obtained in collaborative studies of moisture, protein, and ash determinations have revealed the existence of rather large systematic errors among laboratories. Thus Treloar (1932), in a study of quadruplicate moisture values for one flour determined in 96 laboratories, reported a range of 2.7% in the mean moisture results obtained by different collaborators. The rejection of outlying moisture values, which left a selected group

¹ Paper No. 1998, Scientific Journal Series, Minnesota Agricultural Experiment Station.

of compact distribution, still gave a wide range (0.8%) among laboratories. Davis and Wise (1933), in a study of 2,026 flour-moisture values compiled from collaborators' reports of the Pioneer Section for a five-year period, also found large systematic differences among laboratories; the error distribution of 99% of all the reports covered a moisture range of 0.99%.

It must be emphasized, however, that in both of these studies the moisture methods used were not identical. Davis and Wise (1933) point out that 65% of the collaborators employed the air oven and 35% the vacuum oven, and a wide range of conditions was reported by those using the vacuum oven. Moreover it was pointed out that changes in moisture content in transmitting samples in ointment tins sealed with waterproof tape may have occurred in some instances and contributed to the systematic error.

In contrast to the large systematic errors between laboratories, the precision of the moisture test within laboratories may be regarded as fairly satisfactory. Treloar (1932) found that the average standard error of quadruplicate moisture tests for 96 laboratories was 0.064%, while Davis and Wise (1933) arrived at an average standard error for replicates within laboratories of 0.19%. More recently Eva, Milton, and Geddes (1939) analyzed ten flour samples ranging from 9.7% to 16.6% in moisture content in duplicate by the 130°C air-oven and vacuum-oven methods. The vacuum-oven method gave a significantly higher mean moisture by approximately 0.1% than the air-oven method. The standard errors were 0.02% and 0.05% for the vacuum-oven and air-oven methods, respectively.

In a recent study of the net weight changes and moisture contents of wheat flour at various relative humidities, Anker, Geddes, and Bailey (1942) had occasion to carry out moisture tests by both the vacuum-oven and air-oven methods. As a check on their laboratory findings, the samples were distributed to three commercial laboratories for moisture tests by the air-oven method. These data provide an opportunity for determining the agreement between the vacuum- and air-oven methods and the precision of the air-oven moisture test between and within laboratories. In view of the large systematic errors previously reported in the literature, it seems worth while to record the results of a statistical analysis of these data.

Experimental

The experimental material comprised an 83% patent flour, which yielded 151 samples varying in moisture content from 9.8% to 14.4% as a result of varying the times of exposure in 5-, 10-, and 24 ½-pound sacks to different relative humidities. Four subsamples were taken

from each sack after thorough mixing in a MacLellan batch mixer and placed in air-tight containers. One sample was employed in this laboratory for moisture determinations in triplicate by both the vacuum-oven and 130°C one-hour air-oven methods as described in *Cereal Laboratory Methods* (4th ed., 1941). The remaining three samples were delivered the same day to each of three local commercial mill laboratories which determined and reported their moisture values in duplicate by the 130°C one-hour air-oven method. In most instances the analyses were made the day following sampling. Initially, one of the commercial laboratories used a slightly different technique from the standard air-oven method but 131 of the samples, covering the entire moisture range, were analyzed by the prescribed air-oven method in all four laboratories.

Results

The mean moisture values for each laboratory and method are summarized in Table I, together with the corresponding standard

TABLE I
MEANS AND STANDARD ERRORS FOR MOISTURE DATA

VACUUM AND AIR-OVEN RESULTS FOR ONE LABORATORY—151 SAMPLES		
	Mean %	Standard error (single determination) %
Vacuum-oven method	12.322	0.041
Air-oven method	12.264	0.042
AIR-OVEN RESULTS FOR DIFFERENT LABORATORIES—131 SAMPLES		
Laboratory	Mean %	Standard error (single determination) %
A	12.163	0.051
B	12.082	0.050
C	12.164	0.032
D	12.094	0.040
All	12.126	0.041

errors for single determinations. The mean moisture content of the 151 samples, determined by the vacuum-oven method, was 0.06% higher than that by the air-oven method. This difference was found to be statistically significant and is in line with a similar observation by Eva, Milton, and Geddes (1939). For all practical purposes, however, the agreement between the two methods is very close, and the standard errors are practically identical.

With reference to the air-oven results on 131 samples, a variance analysis of the combined data for all laboratories revealed that the

mean moistures for the four laboratories were significantly different. The interaction between samples and laboratories was also significant. However, the low replicate variance coupled with the large number of degrees of freedom for this variance renders this a very precise test. The range of the laboratory means was only 0.082%. The standard deviation of "within" samples (which includes the variability due to differences between laboratories, the interaction between samples and laboratories, and the replicate error) was 0.093% as compared with a standard deviation of replicates for all laboratories combined of 0.041%. Taking the level of significance as twice the standard error, a difference of 0.2% (2×0.093) between the mean values of two samples analyzed in different laboratories would be significant. However, if the two samples were analyzed in the same laboratory, a difference of 0.1% (2×0.043) would be significant. For practical purposes the precision between and within laboratories is quite satisfactory.

These studies present a much more favorable picture than those reported by Treloar (1932) and Davis and Wise (1933) and show that excellent agreement in air-oven moisture values may be obtained between different laboratories by strict adherence to the prescribed method.

Summary

The vacuum-oven method gave a mean moisture value 0.06% higher than the 130°C one-hour air-oven method in triplicate analyses by one laboratory of 151 flour samples containing from 9.8% to 14.4% moisture. The standard error for each method was 0.04%.

Mean air-oven moisture values of 12.08, 12.09, 12.16, and 12.16% obtained by four laboratories in the analyses of 131 of the above samples were significantly different. For all laboratories combined, the standard deviation for "within" samples was 0.09% as compared with a standard deviation of replicates of 0.04%. Accordingly, a difference of 0.2% in the means of two duplicate determinations of any two samples analyzed in the different laboratories would be significant; if analyzed in the same laboratory a difference of 0.1% would be significant.

These studies show that excellent agreement in air-oven moisture values may be obtained between laboratories by strict adherence to the prescribed method.

Acknowledgments

The authors are indebted to Betty Sullivan, Leslie R. Olsen, and F. C. Collatz for their co-operation in carrying out moisture tests.

Literature Cited

- Anker, C. A., and Geddes, W. F., with Bailey, C. H.
1942 A study of the net weight changes and moisture content of wheat flour at various relative humidities. *Cereal Chem.* 19: 128-150.
- Davis, C. F., and Wise, M.
1933 A five-year summary of the monthly check sample reports of the Pioneer Section of the A. A. C. C. *Cereal Chem.* 10: 203-212.
- Eva, W. J., Milton, Nancy, and Geddes, W. F.
1939 Comparative methods of moisture determination with special reference to the Brabender grinder and oven. *Cereal Chem.* 16: 460-468.
- Treloar, A. E.
1932 The distribution of errors for the determination of protein, moisture, and ash in flour. *Cereal Chem.* 9: 449-472.
- Treloar, A. E., and Harris, J. Arthur
1928 Criteria of the validity of analytical methods used by cereal chemists. *Cereal Chem.* 5: 333-351.

PROTEASE ACTIVITY AND REDUCING MATTER CONTENT
OF WHEAT FLOUR DOUGHS IN RELATION
TO BAKING BEHAVIOR¹

TING SHEN and W. F. GEDDES

Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota

(Read at the Annual Meeting, May 1941)

It has been known for many years that certain oxidizing agents, such as potassium bromate and iodate, ammonium persulfate, chlorine, and nitrogen trichloride, have an "improving action" when used in appropriate amounts in the manufacture of yeast-leavened bread. Relatively minute quantities of these oxidizing agents markedly increase the toughness and stability of the dough and the ultimate effects are observed in the loaf in improved volume, loaf shape, crumb grain, and crumb texture. The importance of these substances has led to much research and considerable speculation concerning their mode of action.

From a review of the early literature by Bailey (1925), it would appear that these flour improvers were generally believed to function by stimulating yeast activity. Geddes and Larmour (1933) and Jørgensen (1935, 1935a) have found that potassium bromate does not influence the rate or the amount of carbon dioxide production in bread doughs. More recently Sullivan, Howe, Schmalz, and Astleford (1940) have reported that this improver depresses gas production very slightly but the differences noted were not regarded as of any significance in relation to its baking effects. Investigators in this field are now generally agreed that the oxidizing improvers pro-

¹ Paper No. 2,000, Scientific Journal Series, Minnesota Agricultural Experiment Station. Condensed from a thesis presented by Ting Shen to the faculty of the Graduate School in partial fulfillment of the requirements for the degree of Master of Science, December, 1940.

duce their principal effects by some action on the gluten proteins which improves the gas-retaining capacity of the doughs, but there is no unanimity of opinion as to the fundamental nature of the reactions involved.

Of the various oxidizing improvers, potassium bromate has been the most extensively studied. In a review paper dealing with various aspects of the bromate baking test, Geddes and Larmour (1933) point out that this reagent has proved particularly useful in the experimental evaluation of wheat strength. The bromate baking results were more highly correlated with protein content than those obtained by the A. A. C. C. basic formula. However, bromate baking response is only in part conditioned by protein content and is influenced by a number of other factors such as the presence of green, frosted, or immature kernels in the wheat mix, the extent of natural aging, whether or not the sample has been heat treated, and the presence of germ constituents.

The early experiments of Geddes (1930) served to emphasize the role of germ constituents in flour baking behavior. The addition of untreated wheat germ to a highly refined flour produced "green" or underfermented characteristics in the baked loaf. Of particular importance, in the light of current theories, was the observation that the addition of the germ produced soft, sticky doughs, the inferior handling properties of which were most noticeable immediately after mixing and that the harmful effects of the germ on the physical properties of the doughs as well as on the volume, external appearance, and crumb grain and texture of the baked loaves decreased with an extension of the fermentation time. The addition of potassium bromate or heat treatment of the germ reduced its detrimental effects and at the same time shortened the fermentation time necessary to produce a loaf possessing the characteristics of normal fermentation. In view of these observations it was pointed out at that time that the deleterious effects of the germ could not be ascribed to the proteases present in the germ. If increased proteolytic activity were responsible for the harmful effects, the dough-handling properties and loaf characteristics would become progressively worse instead of progressively better as fermentation time was extended.

Since heat treatment of the germ, the use of bromate, and longer fermentation time each resulted in improved baking results and gave supplementary effects when combined, Geddes (1930) suggested that these treatments had, in part at least, a common basis through a similar action on certain germ constituents and that the baking improvement resulting from natural aging was probably the result of similar physical or chemical changes. On the basis of Working's

studies (1924, 1928, 1928a) on the injurious effects of phosphatides on gluten quality and his views that the beneficial effects of natural aging and chemical improvers might be due to changes in the solubility of the phosphatides, Geddes suggested that they might prove to be the constituents involved in the common improvement resulting from heat treatment, oxidizing agents, and extended fermentation. No actual experiments were conducted to determine the nature of the substance or substances involved. However, Saunderson and Winkler in 1930 and 1931 (unpublished, reviewed by Geddes and Larmour, 1933) found that natural aging and partial ether extraction of wheat germ brought about a considerable reduction and acetone extraction a lesser reduction in its harmful baking effects. Heat treatment and extraction with these solvents decreased the lipid phosphorus content and the evidence, though indirect, pointed to a close relation between phosphatide content and the baking behavior of germ-flour mixtures. Sullivan and Near (1933) found that the lipid phosphorus of germ rapidly decreased upon storage, particularly at high moisture contents.

The studies of Rich (1934), Sullivan, Near, and Foley (1936, 1936a), Bull (1937), Sullivan, Howe, and Schmalz (1936, 1937), Sullivan and Howe (1937), and Sullivan, Howe, Schmalz, and Astleford (1940) clearly showed that the phosphatides or other lipid materials were not responsible for the injurious effects of wheat germ or for the beneficial effects of oxidizing improvers. The harmful constituents were found to be soluble in water rather than in lipid solvents and ether-extracted flours will give a positive baking response to oxidizing agents. Sullivan and her co-workers identified the presence of glutathione in wheat germ, observed that it had a decidedly harmful effect upon flour baking properties, and presented evidence that its oxidation is in large measure responsible for the beneficial effect of heat treatment on wheat germ and germ-containing flour streams. These studies have directed attention to the importance of sulfhydryl compounds and oxidation-reduction phenomena in relation to flour improvement. The lipids do, however, appear to play a role in the changes in baking quality due to natural aging. It is well known that flour acidity increases on aging and Kozmin (1935), Barton-Wright (1938) and others have shown that the colloidal behavior of gluten is materially influenced by unsaturated fatty acids. In view of the independent discovery by Balls and Hale (1940) and Sullivan, Howe, Schmalz, and Astleford (1940) of a sulfur-containing constituent in flour lipid extracts, the lipids may eventually be found to play some part in oxidation-reduction phenomena during dough fermentation. Reference must also be made to the suggestion put forward by Bungenberg de Jong (1938) that gluten interacts with lecithin, thereby

making it possible for hydrophobic substances such as fats and sterols to be bound to the protein. The solvation of this complex is influenced by unsaturated fatty acids and the system is sensitive to oxidation-reduction processes.

Jørgensen (1935, 1935a, 1936, 1938), in experiments in which the increase in water-soluble nitrogen upon autolysis of flour suspensions was determined, reported that wheat flour contains powerful but latent proteolytic enzymes which are activated by glutathione and yeast water and inhibited by potassium bromate and iodate (but not by potassium chlorate) and ascorbic acid. Likewise, potassium bromate and iodate, but not potassium chlorate, also inhibited the protein-splitting action of papain, wheat germ, pineapple, liver, and flour proteases when acting on gelatin. Since potassium bromate and iodate are flour improvers, while potassium chlorate is not, Jørgensen proposed the theory that potassium bromate and analogous oxidizing agents exert their beneficial effects on baking quality by inhibiting more or less completely the flour proteinases, which are rendered active by the yeast and glutathione, thereby diminishing the breakdown of the gluten proteins during fermentation and improving the gas-retaining capacity of the doughs. Likewise the important studies of Balls and Hale (1935, 1936, 1936a, 1938) and of Hale (1939) on the proteinase enzymes of wheat bran and wheat flour led them to conclude that the inactivation of wheat proteinase by oxidizing improvers adequately explains their action as bread improvers when added to fermenting doughs.

The proteolytic theory of Jørgensen and of Balls and Hale was opposed by Read and Haas (1937, 1938, 1939), who repeated certain of Jørgensen's extraction studies and pointed out that the ratio of potassium bromate to flour employed by him greatly exceeded the amount used in baking practice. When used at commercial levels they secured no significant repression of proteinase activity; moreover they reported that the proteinase of malted wheat flour was not as readily inhibited by bromate as papain and bromelin. In answer to Read and Haas, Jørgensen (1939) pointed out that because of the wider flour-water ratio in the extraction tests, larger amounts of bromate would be required to produce effects corresponding to those in dough; moreover, in a fermenting dough, yeast activates the flour proteinases so that the amounts of bromate necessary to show a reduction in nitrogen solubility in extraction tests cannot be directly compared with the quantities effective in bread doughs. Further experiments were then reported by Jørgensen which indicated that, when due consideration is given to these points, dosages of bromate corresponding to those used in baking reduce the solubility of the

flour nitrogen. Flohil (1936) gave extensive evidence of the depressing action of flour improvers on proteolytic activity. He found that wheat germ was exceedingly rich in proteases and attributed its harmful effects on baking value to this fact, pointing out that, in general, the response to improvers increases with increasing germ content.

It must be clearly recognized, as has been pointed out by Sullivan, Howe, Schmalz, and Astleford (1940), that the proteinase theory is not based on direct evidence but rather on an interesting parallelism between the influence of certain reducing and oxidizing agents on the activity of flour proteases and the general effects of these substances on flour baking properties. Reducing agents such as cysteine and reduced glutathione stimulate flour protease activity and have a detrimental effect on gluten quality and flour baking properties, whereas oxidizing agents, such as nitrogen trichloride, persulfates, bromates, and iodates, have an inhibitory effect on flour protease activity and are flour improvers; on the other hand, potassium chlorate is essentially without influence on either protease activity or flour baking properties. Although many investigators have shown that the addition of proteases to flour results in excessive dough slackening and serious injury to baking properties, such experiments do not prove that proteolytic activity is, in itself, of any particular significance in relation to the baking behavior of normal flours. This theory has served a useful purpose in stimulating interest in the complicated problem of the mode of action of oxidizing and reducing agents in fermenting doughs but further progress demands that its limitations be generally recognized. The theory is not in accord with the fact that the lower the grade of flour milled from any particular wheat, the longer, in general, is the fermentation time required to produce an optimum loaf. Also, it fails to explain satisfactorily the harmful baking effects of excess bromate.

Ford and Maiden (1938) carried out farinograph studies on flour doughs to which glutathione and papain, respectively, had been added. Glutathione had a direct and immediate softening effect, whereas papain had, as well as an immediate action, a very marked delayed softening effect of the type one would expect as a result of increased protease activity. On the other hand the softening of the doughs containing glutathione did not increase with time to the extent that would be expected if it functioned mainly as an activator of flour proteases. In the light of these results, Ford and Maiden believe that the baking effects of glutathione are due primarily to a direct action on the flour proteins themselves and not to flour protease activation.

Upon testing doughs with the extensograph at varying intervals of time after molding, Munz and Brabender (1940) found that the extensibility (E) of the doughs decreased and the resistance to extension (F) increased with rest time. Corresponding bromated and non-bromated doughs gave similar curves immediately after mixing, but after a period of rest the tightening effect of the bromate became evidenced by a substantial increase in the F/E ratio. When potassium persulfate was used instead of bromate, a more immediate effect was registered. In the instance of flours which gave a strong positive response to bromate, the area under the extensogram curve was markedly increased. The extensibility of doughs containing papain and cysteine was greater and the resistance to extension less than for normal doughs tested at corresponding time intervals. The differences between these values for the normal and treated doughs increased with time, thus providing physical evidence in support of the inhibitory influence of bromate on the action of papain and similar proteolytic enzymes.

Baker and Mize (1937, 1939) have carried out tests on the effects of mixing doughs in vacuum and in the presence of such gases as air, oxygen, nitrogen, and hydrogen, with and without the addition of inorganic peroxides and potassium bromate. They found that doughs could be mixed for prolonged periods in vacuum or inert gases without deterioration. However, deterioration occurred when oxygen or inorganic oxidizing agents were added to the doughs. The action of bromate was found to be promoted by mechanical mixing. In the absence of both mechanical action and yeast fermentation, bromate had little effect.

Freilich and Frey (1939, 1939a, 1939b) found that potassium bromate produced effects in addition to and apparently different from direct inhibition of proteolytic activity. Excessive amounts of bromate produced great decreases in loaf volume, the "excess bromate effect" being greater for any given dosage level with longer fermentation time. They point out that if the effect of bromate were merely to inhibit protease activity, there should be no further effects when sufficient amounts of the oxidizing agent to accomplish this purpose have been added. It was found that the undesirable effects produced by excessive amounts of bromate, or by prolonged fermentation, could be eliminated by remixing the doughs after fermentation. These investigators also carried out numerous experiments in which the doughs were mixed in oxygen. Mixing in oxygen reduced the harmful effects of added pepsin, wheat germ, cysteine, and glutathione, and the production of amino nitrogen was markedly retarded.

Sullivan and her associates have made important contributions in this field. Recently Sullivan, Howe, Schmalz, and Astleford (1940) have reviewed the literature and presented additional studies on the fundamental nature of the action of oxidizing agents as flour improvers. They concluded that the starch, lipids, sugars, and diastatic enzymes of the wheat flour are of secondary importance and pointed out the insufficiency of the proteolytic theory to explain all the known facts. They believe that the detrimental effect of glutathione and other sulfhydryl compounds in relation to baking quality of flour is due mainly to their direct action on the colloid properties of the gluten and conclude that the effect of oxidizing and reducing agents on flour can be best explained, with the facts at present available, on the basis of their direct action on the sulfhydryl linkage of the flour proteins.

Freilich and Frey (1940), employing an iodine titration method developed by Freilich (1941), determined the reducing matter in low-grade, clear, straight-grade, and patent flours milled from both Texas and northwestern wheats. Protease activity was also determined by a formol titration procedure on doughs mixed from these flours after standing 24 hours at 86° F. No yeast was added in mixing the doughs since it was found that the amino nitrogen produced by proteolytic activity was utilized by the yeast. The activity of any wild yeasts which might have been present was inhibited by octyl alcohol which was shown to have no effect on protease activity. The results of these determinations were correlated with the results of baking tests conducted on doughs mixed in oxygen, and nonbromated and bromated doughs mixed in nitrogen, respectively. The protease activity and reducing-matter contents of the various flour grades were found to be positively related. Similarly protease activity and reducing-matter contents were each positively correlated with the increase in loaf volume due to oxidation. These workers suggest that these two factors may be of about equal importance as indicators of baking improvement resulting from the use of oxygen or oxidizing agents.

The effect of skim-milk solids on baking behavior is of interest in connection with the action of oxidizing agents. The experiments of Greenbank, Steinbarger, Deysher, and Holm (1927), Grewe and Holm (1928), Skovholt and Bailey (1931, 1932), Johnson and Ward (1936), and others, show quite definitely that the "baking quality" of milk may be improved by suitable heat treatment. Ofelt and Larmour (1940) have made the important observation that the addition of skim-milk solids not only increases the bromate requirement for optimum baking results but also has a buffering effect, increasing the tolerance towards excessive dosages. Treatment of two flours with the protease activator cysteine monochloride did not accentuate their

differential response to bromate, indicating that the protease content of the flours was not responsible for the differences. The addition of cysteine produced a very marked and almost instantaneous effect on the physical properties of the doughs which, according to these authors, was too rapid to be attributed to enzyme action and appeared to have the characteristics of a colloidal effect. Eisenberg (1940) has confirmed the buffering effect of milk solids on bromate.

Investigations of the thiol groups of proteins by Hopkins (1930, 1930a), Mirsky and Anson (1935, 1936), Greenstein (1938, 1939, 1939a) and others, reveal that the heat denaturation of proteins is accompanied by an increase in sulfhydryl groups. Gould and Sommer (1939, 1939a) have shown that normal milk gives a negative nitroprusside test, but when it is heated to 90° C a faint positive reaction is obtained. Moreover, a distinct increase in the intensity of the color reaction upon treatment with cyanide was obtained with milks heated to temperatures of 76°–80° C, indicating greater ease of reduction of the sulfur linkages as a result of heat treatment. The development of the cooked flavor upon heating was associated with the formation and liberation of hydrogen sulfide, and a lowering of the oxidation-reduction potential. These observations suggest that the improvements in the "baking quality" of milk resulting from heat treatment, and the effect of milk on bromate requirement and tolerance, may be related to changes in the sulfur linkages.

Recently Ziegler (1940, 1940a, 1940b, 1941) has published a series of papers relative to glutathione oxidation and the effects of the reduced and oxidized forms on dough properties as measured by Chopin extensimeter and farinograph tests.

In aqueous solution at temperatures below 40°C, reduced glutathione (GSH) was slowly oxidized by potassium bromate, but very rapidly oxidized by iodine at pH 4.5–5.6. Farinograph studies indicated that the oxidation of GSH by bromate was not catalyzed by yeast or the constituents of a nonfermenting dough. While GSH additions to flour doughs yielded soft sticky doughs and lowered the loaf volume, very small amounts of GSH had a beneficial effect. Sodium chloride in proportions used in baking partially protected the gluten against the harmful action of GSH. Oxidized glutathione was apparently reduced again in nonfermenting doughs, probably due to the accumulation of reducing sugars. Oxidation of aqueous GSH solutions with ammonium persulfate required more than thirty times greater concentration than of potassium bromate while only about three times the concentration is needed for flour improvement, indicating that the improving action of persulfates may be due to factors other than GSH oxidation. A study was also made of the effect on

gluten swelling properties and flour baking behavior of various substances known to be involved in, or influencing, respiratory rate. Succinic acid, fumaric acid, and adrenaline, which increased respiration, improved baking quality, although only adrenaline in the presence of a trace of copper actually inhibited proteolysis.

Iodine titration and gluten swelling studies showed that the flour-improving effect of bromate, iodate, persulfate, and dehydroascorbic acid could at least partly be explained by their ability to oxidize glutathione. These substances also inhibited the breakdown of gluten by commercial papain, but Ziegler pointed out that as the papain preparation may have contained an activator, these results cannot be interpreted as proving that the oxidizing agents act directly on the enzyme itself. Ziegler interpreted his studies as being in line with the Jørgensen theory.

Sullivan, Howe, Schmalz, and Astleford (1940) have pointed out that the amount of bromate required to produce optimum response is greater with brake than with normal doughs and have confirmed the observations of Freilich and Frey (1939a) that overtreated doughs can be brought back to normal on remixing. Neither this excess effect of oxidizing agents nor its amelioration by purely physical means can be satisfactorily explained by the theories of Jørgensen and of Balls and Hale. Sullivan and coworkers also point out that different flour improvers produce somewhat different effects on flour doughs; for example, iodate exhibits a beneficial action on dough much earlier in the fermentation than does bromate, which is in line with the observation that iodate does not require as low an acidity to function as an oxidizing agent.

This general review of the literature indicates that the problems involved in the mechanism of the action of oxidizing agents in fermenting doughs are by no means simple. It is clear, however, that their baking effects are due primarily to some action involving the gluten proteins, but on purely theoretical grounds there are several ways in which modifications in the properties of the flour proteins could be brought about. These include a direct action on the sulfur linkages of the gluten and indirect effects which might be brought about by a number of possible ways. The colloidal character of the gluten might be influenced indirectly with or without oxidation of the gluten itself by an intermediate substance which has been oxidized by the flour improver. On the other hand, the oxidizing improvers could function by decreasing proteolytic activity either by direct oxidation of the proteinase enzyme itself or by conversion of an activator, through oxidation, into an inactive form.

The question arises as to how many of these possible mechanisms are actually functioning when oxidizing agents are added to fermenting doughs, and if several are operative, which of these are of primary importance. The literature at present available clearly indicates that more than one mechanism is involved. The inhibition of protease activity by oxidizing improvers has been well established, and flours of high protease activity and reducing-matter content give marked baking responses to oxidizing improvers. However, the very rapid effect of glutathione, wheat germ, and other reducing substances on dough properties, the lessening of their harmful effects upon extension of the fermentation time, coupled with the "excess bromate effect" and its amelioration by purely physical means (remixing after fermentation), strongly indicate that proteinase activity is not of primary importance in relation to the baking effects of oxidizing agents. Moreover, if active proteolysis taking place during fermentation has a marked degrading effect on the physico-chemical properties of the flour proteins one would anticipate that the low-protein, weak flours would show the greatest response to oxidizing improvers. The fact that the high-protein flours give the greatest response can apparently be interpreted only in terms of the proteolytic theory by assuming that such flours have a higher protease and/or reducing-matter content.

On the basis of our present knowledge, two other possible mechanisms seem to be much more attractive than the proteinase theory: (1) the oxidation of glutathione and other reducing substances in the dough which exert a direct effect on the colloidal properties of the flour proteins rather than an indirect effect through the medium of proteinase activation, and (2) the direct oxidation of the sulfhydryl linkages of the flour proteins. Sullivan, Howe, Schmalz, and Astleford (1940) have proposed two explanations of improver action based on these possibilities. They favor the theory that dough fermentation, physical manipulation of the doughs, and oxidizing and reducing agents bring about changes in the sulfur linkages of the gluten proteins. In accord with observations that protein denaturation results in marked changes in the physical properties of various proteins and is accompanied by an increase in RSH groups, it is suggested that during fermentation, alteration or cleavage of R-S-S-R linkages may take place, with the production of, for example, RSH groups, some of which may be oxidized by air or oxidizing improvers, the rate of oxidation being dependent upon their degree of dissociation and the oxidation potential of the fermentation. This theory is particularly attractive since it provides a common basis for explaining flour improvement by physical and chemical means. In the light of the above theory it would be of interest to follow the changes in the reducing-

matter content of fermenting doughs and, in particular, to determine the influence of potassium bromate on the reducing matter levels. In the studies of Freilich and Frey (1940), proteolytic activity was determined in nonyeasted doughs (with the addition of sufficient octyl alcohol to prevent any activity of wild yeasts) after a fixed time, and reducing matter was determined in the flour. The objectives of the present study were to extend their work by determining the amino nitrogen and reducing-matter content of actual fermenting doughs baked with and without bromate for varying fermentation times and to ascertain whether any relation exists between the extent of proteolysis and the reducing matter content of the doughs at the end of the proof period, and (1) the volume of the baked loaf and (2) the loaf volume response to bromate.

Experimental

The experimental material consisted of three grades of flour: short patent, fancy clear, and low grade from the same commercial mill mix of hard red spring wheat. The protein and ash values of the flours, which were all untreated, are given in Table I.

TABLE I
PROTEIN AND ASH CONTENT OF FLOURS EMPLOYED

Flour grade	Crude protein ¹	Ash ¹
	%	%
Short patent	12.5	0.42
Fancy clear	15.8	0.72
Low grade	16.3	1.02

Each flour was baked with additions of nil, 0.001, 0.002, 0.004, and 0.006% potassium bromate with fermentation times of 1.5, 3.0, and 4.5 hours respectively. Reducing matter and amino nitrogen content were determined after mixing and again at the end of the proof period on doughs made without and with 0.004% potassium bromate for each of the above fermentation times. These determinations were made on each of the respective doughs with and without the addition of octyl alcohol.

Experimental baking methods: The basic formula (to which the required additions of potassium bromate were made) was as follows: flour (15% moisture basis) 100 g, yeast (Fleischmann's) 3 g, sucrose 5 g, sodium chloride 1 g, and sufficient distilled water to yield a dough of the desired consistency. Three hundred grams of flour was mixed with the proportional quantities of the other dough ingredients in a

¹ Expressed on 13.5% moisture basis.

Hobart-Swanson mixer for two minutes and scaled into three portions of 150 g each for the three different fermentation times. The doughs were fermented at 30°C with the following schedule for punching and molding:

Total fermentation time	Time after mixing			
	First punch	Second punch	Third punch	Molding
<i>hrs</i>	<i>min</i>	<i>min</i>	<i>min</i>	<i>min</i>
1.5	65	nil	nil	90
3.0	105	155	nil	180
4.5	120	195	245	270

All doughs were proofed 55 minutes at 30°C and baked for 25 minutes at approximately 230°C. The baking tests were carried out in duplicate, the replicate loaves being baked on different days. Loaf volume was measured approximately two hours after removing from the oven and the loaves were scored the following day.

Determination of amino nitrogen and reducing matter in doughs: Since soluble nitrogen compounds are utilized by actively fermenting yeast, it is necessary, as pointed out by Freilich and Frey (1940), to inhibit yeast fermentation or to make the tests on doughs fermented to the same extent. These workers have shown that yeast activity can be inhibited by a suitable concentration of octyl alcohol—a reagent which was found to be without influence on proteolytic activity. Since in our studies it was desired also to determine reducing matter in the doughs at the end of the proof period, a preliminary experiment was carried out to ascertain the effect of yeast and octyl alcohol on the level of reducing matter. Doughs were prepared from the low-grade flour by the formula employed for the experimental baking tests as follows: (1) basic dough, (2) basic dough + octyl alcohol, (3) basic dough without yeast, (4) basic dough without yeast + octyl alcohol. Octyl alcohol, where required, was used at the rate of 0.3 ml per 100 g of flour, the concentration which was found by Freilich and Frey (1940) to be effective in inhibiting yeast fermentation.

Determinations of amino nitrogen and reducing matter were made for each of the above doughs 1.5, 3.0, and 4.5 hours, respectively, after mixing. For each of these times, doughs were prepared with 200 g of flour and the other ingredients (with or without yeast and/or octyl alcohol as required for the different series outlined above) as described for the experimental baking tests. Two 150-g doughs were scaled, punched, fermented, and proofed in the manner employed in carrying out the baking tests. After proofing, the duplicate doughs were placed together in a Waring Blendor, the glass container of which

was modified in a manner similar to that described by Freilich and Frey (1940) by reducing its capacity and grinding the top edges and fitting thereto a glass plate through which passed two glass tubes equipped with stopcocks.

Two grams of sodium chloride and 500 ml of distilled water were added, the glass cover placed in position, nitrogen gas passed in, and the dough disintegrated by operating the Blendor for 2.5 minutes. This treatment resulted in a uniform suspension. A portion of the suspension was centrifuged and the amino nitrogen determined in 10 ml of the supernatant liquid by a modification of the Sorenson formol titration procedure suggested by Samuel (1934) using $N/14$ NaOH solution. This procedure, fully described in *Cereal Laboratory Methods* (4th ed., 1941), involves the use of phenol red indicator and titration to pH 8.0 as determined by matching against a buffer solution of this pH in a comparator block. By centrifuging at high speed for 10 minutes to secure a reasonably clear extract and titrating immediately after addition of the indicator, very satisfactory end points were obtained. The results were calculated in terms of milligrams of amino nitrogen in a dough weight equivalent to 100 g of flour (15.0% moisture basis).

For the determination of reducing matter, the method recently described by Freilich (1941) was employed. This is based upon oxidation of an extract of the flour with iodine at approximately pH 3.0 from which the proteins have been precipitated by sulfosalicylic acid and sodium chloride. Half of the suspension prepared from the dough was allowed to stand in a stoppered bottle for 45 minutes, and 10 ml of 60% sulfosalicylic acid solution added with slight shaking. After 15 minutes the mixture was centrifuged in a glass-stoppered bottle and the extract filtered through folded paper. Immediately after filtering 5 ml of 0.005*N* iodine solution was added to 50 ml of the clarified extract and the solution back titrated with 0.005*N* sodium thiosulfate solution using 2 ml of 1% starch solution as indicator. The results were expressed as ml 0.001*N* iodine consumed by a quantity of the dough extract equivalent to 100 g of flour (15.0% moisture basis). Experiments in which the use of nitrogen gas was eliminated and the titrations made at different times after filtration of the centrifuged extract confirmed the observations of Freilich (1941) that there is a gradual loss of reducing matter upon exposure to air. Accordingly, nitrogen gas was always employed and the same time schedule was adhered to from sample to sample in order to secure as comparable results as possible.

The values recorded in Table II confirm the findings of Freilich and Frey (1940) regarding the necessity of inhibiting fermentation if

TABLE II

EFFECT OF YEAST AND FERMENTATION (OR REST) TIME ON AMINO NITROGEN AND REDUCING MATTER CONTENT OF DOUGHS MADE WITH LOW GRADE FLOUR

Fermentation ¹ (or rest) time	Basic dough		Basic dough without yeast	
	Without octyl alcohol	With octyl alcohol	Without octyl alcohol	With octyl alcohol
AMINO N PER DOUGH WEIGHT EQUIVALENT TO 100 G FLOUR				
hrs	mg	mg	mg	mg
1.5	22.1	28.2	22.1	21.3
3.0	7.1	31.7	24.4	22.3
4.5	6.1	33.5	25.6	22.8
REDUCING MATTER (ML) 0.01 <i>N</i> IODINE PER DOUGH WEIGHT EQUIVALENT TO 100 G FLOUR				
hrs	ml	ml	ml	ml
1.5	10.3	14.0	8.6	8.5
3.0	11.0	13.9	9.9	9.2
4.5	9.5	14.4	10.5	11.1

¹ Proofing time 55 minutes is not included in the times recorded.

the amino nitrogen content of the doughs after varying rest times is to be taken as a measure of proteolytic activity. In general, the presence of yeast increased the reducing matter content of the dough, particularly when octyl alcohol was added. In view of the results of these preliminary experiments it was deemed advisable to determine amino nitrogen and reducing matter content of basic and bromated yeast doughs made both with and without the addition of octyl alcohol. The effect of bromating was ascertained only for the 0.004% dosage. The determinations were made by the methods already described on the doughs immediately after mixing and also after 1.5, 3.0, and 4.5 hours of fermentation.

Results

The mean loaf volumes for each flour, bromate dosage, and fermentation time are recorded in Table III and graphically depicted by the histograms given in Figure 1. The data for the amino nitrogen and reducing matter content of the basic and bromated doughs (4 mg level only) are given in Table IV and Figure 2.

The baking results confirm previous observations (Geddes and Larmour, 1933) regarding the greater response and greater tolerance of fancy clear and low-grade flours, to bromate as compared with the patent flour. At 1.5 hours of fermentation all three flours gave a

TABLE III

LOAF VOLUME DATA FOR VARIOUS BROMATE DOSAGES AND FERMENTATION TIMES

Bromate per 100 g flour mg	Mean loaf volumes with fermentation time in hours		
	1.5 hrs	3.0 hrs	4.5 hrs
PATENT FLOUR			
0	563	595	583
1	668	585	558
2	670	580	485
4	695	485	360
6	685	425	350
FANCY CLEAR FLOUR			
0	590	615	565
1	668	793	755
2	708	915	685
4	963	663	470
6	980	668	413
LOW-GRADE FLOUR			
0	555	545	538
1	590	643	670
2	615	758	618
4	718	600	405
6	810	510	380

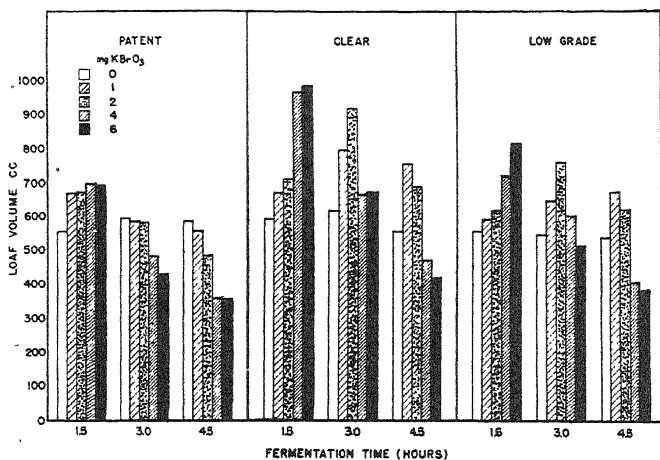


Fig. 1. Effect of bromate dosage and fermentation time on the loaf volumes of patent, clear, and low-grade flours.

positive response to bromate, the response increasing with the bromate dosage, except in the case of the patent flour which gave an optimum response at the 4-mg level. At 3.0 and 4.5 hours of fermentation time the positive responses in loaf volume were limited to the lower bro-

TABLE IV
AMINO NITROGEN AND REDUCING MATTER CONTENT OF DOUGHS

Treatment per 100 g flour		Amino N per 100 g flour— fermentation (or rest) time in hours ¹				Reducing matter 0.01N iod. 100 g flour—fermentation time in hours: ²			
Bro- mate	Octyl alcohol	0	1.5	3.0	4.5	0	1.5	3.0	4.5
mg	ml	mg	mg	mg	mg	ml	ml	ml	ml
PATENT FLOUR									
0	0	8.6	1.5	0.3	0.3	2.3	5.4	6.7	5.7
4	0	7.6	0.0	0.0	1.3	.6	3.6	4.3	4.4
0	3	18.0	19.5	22.1	22.8	4.2	5.1	5.7	5.4
4	3	18.5	20.8	21.3	23.3	3.4	2.0	1.9	2.7
FANCY CLEAR FLOUR									
0	0	13.9	6.1	2.8	2.3	3.5	6.1	6.2	7.0
4	0	10.4	1.8	1.5	3.0	3.1	5.5	5.1	4.5
0	3	22.0	25.6	29.2	29.7	6.6	8.4	9.8	10.3
4	3	22.3	25.4	27.1	28.4	5.7	6.8	6.6	6.5
LOW-GRADE FLOUR									
0	0	15.5	22.1	8.6	6.1	5.9	10.3	11.0	9.5
4	0	9.4 ²	3.0	3.0	8.1	5.3	6.8	7.3	3.4
0	3	24.9	28.2	31.7	33.5	9.8	14.0	13.9	14.4
4	3	23.1	27.9	29.4	32.2	8.1	8.8	9.8	10.1

¹ Proof period of 55 minutes not included in times given.

² Single determination only.

mate dosages in both low-grade and fancy clear flours while all bromate treatments gave negative responses with patent flour; thus the greater the bromate dosage, the shorter the fermentation time required to give optimum baking results. The handling properties of the doughs changed materially as the fermentation time was extended; those made with high bromate dosages gradually became "tough" and "solid."

The amino nitrogen content of the doughs at mixing time (zero hours of fermentation) increased with decreasing flour grade. In all three grades of flour both the basic and bromated doughs showed a gradual increase in amino nitrogen content with increasing "rest time" when yeast activity was inhibited by addition of octyl alcohol. In the presence of yeast fermentation, there was, in general, a pronounced drop in amino nitrogen with increasing fermentation time as a result of the utilization of amino nitrogen by the active yeast cells.

There was always a somewhat lower content of amino nitrogen in the bromated as compared with the corresponding nonbromated doughs

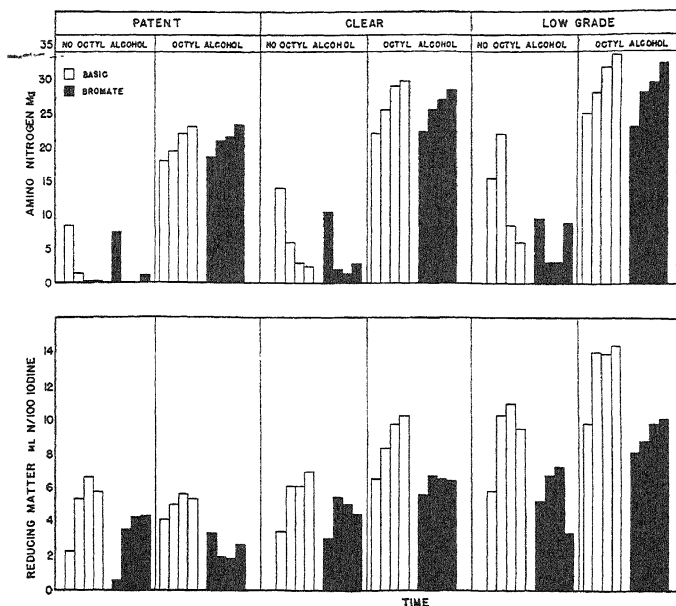


Fig. 2 Amino nitrogen and reducing matter content of basic and bromated (4 mg) doughs made with and without the addition of octyl alcohol after various resting or fermentation times. From left to right, the bars in each section represent the values determined on doughs after 0, 1.5, 3.0, and 4.5 hours, respectively.

containing active yeast but in doughs containing octyl alcohol the apparent depressing effect of bromate was very slight.

The reducing-matter content increased with decreasing flour grade and with the exception of the patent flour the amount of reducing matter was uniformly higher whenever yeast activity was inhibited by octyl alcohol. With a few exceptions the reducing-matter content increased with fermentation. In every case the reducing matter of corresponding doughs was very much less whenever bromate was added, the decrease due to bromate being greater the larger the quantity of reducing matter present.

Discussion

In comparing these results with those obtained by Freilich and Frey (1940), it must be emphasized that they determined reducing matter in flours and not in doughs after varying fermentation times; moreover, they determined amino nitrogen in doughs allowed to ferment or to rest (octyl-alcohol-treated doughs) for 24 hours. In the present study, however, both reducing matter and amino nitrogen

were determined on doughs at mixing and at the end of the proof period after 1.5, 3.0, and 4.5 hours of fermentation or resting time. The data for amino nitrogen and reducing matter content are of particular interest because they represent the levels at the end of the proof period under actual baking conditions.

The amino nitrogen values fully confirm the observations of Jørgensen (1935, 1935a, 1936) that bromate exerts a greater inhibitory effect on proteolytic activity when active yeast is present, as judged by the differences in amino nitrogen content at corresponding fermentation times. In doughs containing octyl alcohol, which inhibits fermentation, the results are not entirely consistent but in general indicate a slight depressing effect due to bromate.

Unfortunately the utilization of amino nitrogen by actively fermenting yeast renders it impossible to use the amino nitrogen values after varying fermentation times as a measure of the proteolysis which has occurred. In the instance of the doughs treated with octyl alcohol, amino nitrogen increased with time, and because of the apparent stimulating effect of the yeast it may be assumed that greater proteolysis occurred in the fermenting doughs. Accordingly the relative increase in amino nitrogen content of the basic and bromated octyl-alcohol-treated doughs from the time of mixing may be regarded as minimum values for proteolytic activity. These are recorded in Table V along with the basic and bromate volumes and reducing matter contents for the fermenting doughs. The differences between the corresponding basic and bromate data for the three variables are also recorded.

It is of interest to note that patent flour which has the lowest proteolytic activity and reducing-matter content has the lowest bromate response of the three flours. However, considering the data as a whole, one could not predict the behavior of the flours toward bromating or toward increased fermentation from a knowledge of the amount of proteolysis or reducing-matter content of the basic doughs at a given time after mixing. Bromate and fermentation have a supplementary effect upon loaf volumes, resulting in overdevelopment of the doughs when heavy bromate treatment is combined with extended fermentation, yet bromate reduces the extent of proteolysis and decreases the reducing-matter content, whereas lengthening of the fermentation results in increased proteolysis and also in general in an increase in reducing-matter content. The percentage depression in reducing matter due to bromate is much greater than the indicated percentage depression in proteolysis but it must be remembered that the amino nitrogen values do not represent the conditions in actual fermenting doughs.

TABLE V

EFFECT OF BROMATE TREATMENT (0.004%) AND FERMENTATION ON LOAF VOLUMES AND REDUCING MATTER CONTENT; EFFECT OF BROMATE AND RESTING TIME ON PROTEOLYTIC ACTIVITY

Fermentation time <i>hrs</i>	Loaf volume			Proteolysis—amino N per 100 g flour			Reducing matter—0.01N I ₂ per 100 g flour		
	Bromate	Basic	Response	Bromate	Basic	Response	Bromate	Basic	Response
<i>hrs</i>	<i>cc</i>	<i>cc</i>	<i>cc</i>	<i>mg</i>	<i>mg</i>	<i>mg</i>	<i>ml</i>	<i>ml</i>	<i>ml</i>
PATENT FLOUR									
1.5	695	563	+132	1.5	2.3	-0.8	3.6	5.4	-1.8
3.0	485	595	-110	4.1	2.8	+1.3	4.3	6.7	-2.4
4.5	360	583	-223	4.8	4.8	0.0	4.4	5.7	-1.3
FANCY CLEAR FLOUR									
1.5	963	590	+373	3.1	3.6	-0.5	5.5	6.1	-0.6
3.0	663	615	+48	4.8	7.2	-2.4	5.1	6.2	-1.1
4.5	470	565	-95	6.1	7.7	-1.6	4.5	7.0	-2.5
LOW-GRADE FLOUR									
1.5	718	555	+163	4.8	3.3	+1.5	6.8	10.3	-3.5
3.0	600	545	+55	6.3	6.8	-0.5	7.3	11.0	-3.7
4.5	405	538	-133	9.1	8.6	-0.5	3.4	9.5	-6.5

¹ The values were obtained by subtracting the amino nitrogen levels in the octyl alcohol treated at mixing times from those at specified stages in the fermentation.

Caution must be observed in making broad generalizations from biochemical studies of this nature, especially as there is some doubt as to the adequacy of the methods. Several workers have pointed out that significant changes in the colloidal properties of the flour proteins, as reflected in dough behavior, may take place with little measurable increase in amino nitrogen. Moreover this determination measures peptidase (if present) as well as proteinase activity. In evolving a workable theory of bromate action it would be desirable to determine whether there is a direct proportionate relationship between proteinase-activity depression and reducing-matter decrease by bromating. This requires a method which is specific for proteinases and applicable to fermenting doughs.

The method for reducing matter also leaves much to be desired in view of the difficulty of preventing oxidation during the determination. Information is needed as to precisely what substances in dough are contributing to the values obtained. There is also the question as to whether the determination of reducing matter at pH 3.0 (approximately) yields results which would parallel those which would be

obtained at the higher pH values of fermenting doughs, if consistent results could be secured within such pH ranges. Accepting the results at their face value, they indicate a definite increase in RSH compounds with time in the instance of nonfermenting doughs, made by the basic formula. In general there is also an increase in RSH in the fermenting doughs made by this formula up to three hours of fermentation. It must be noted, however, that the nonfermenting doughs were rendered nonfermenting by octyl alcohol, which may have modified the permeability of the yeast cells and permitted the diffusion of yeast glutathione into the dough solution. It does not appear that reducing sugars, which would accumulate in the nonfermenting doughs, would contribute to the iodine titration at the low pH employed.

In view of the limitations discussed above, the authors prefer not to draw conclusions as to the mechanism of bromate action in dough fermentation. The complete elucidation of this problem must await the development of more satisfactory techniques.

Summary

Baking tests on short patent, fancy clear, and low-grade hard red spring wheat flours made with additions of nil, 0.001%, 0.002%, 0.004%, and 0.006% of potassium bromate at fermentation times of 1.5, 3.0, and 4.5 hours showed the loaf-volume response and tolerance to bromate to be greatest for the fancy clear and low-grade flours. For each flour the bromate response decreased with extension of the fermentation time.

Amino nitrogen and reducing-matter content of the doughs at mixing time increased with decreasing flour grade.

When yeast fermentation was inhibited by octyl alcohol, proteolytic activity of the doughs, as measured by the increase in amino nitrogen from mixing to the end of the proof, increased with fermentation time and was only slightly depressed by bromate. Proteolytic activity increased with decreasing grade. In actively fermenting doughs amino nitrogen was utilized by the yeast, but bromate considerably depressed proteolytic activity as indicated by the lower amino nitrogen levels at corresponding fermentation times.

Reducing-matter content of nonfermenting doughs (octyl-alcohol-treated), increased more with time than that of fermenting doughs. Bromate had a marked depressing effect which was more pronounced the longer the fermentation.

In view of the limitations of the analytical procedures employed, no conclusions are drawn as to the mechanism of bromate action in dough fermentation.

Literature Cited

- Bailey, C. H.
1925 The chemistry of wheat flour. Chemical Catalog Company, N. Y.
- Baker, J. C., and Mize, M. D.
1937 Mixing doughs in vacuum and in the presence of various gases. *Cereal Chem.* 14: 721-734.
1939 The relation of mechanical stirring to sponge doughs. *Cereal Chem.* 16: 294-295.
- Balls, A. K., and Hale, W. S.
1935 Proteolysis in flours. *J. Assoc. Off. Agr. Chem.* 18: 135-140.
1936 Proteolytic enzymes of flour. *Cereal Chem.* 13: 54-60.
1936a Further studies on the activity of proteinase in flour. *Cereal Chem.* 13: 656-664.
1938 The preparation and properties of wheat proteinase. *Cereal Chem.* 15: 622-628.
1940 A sulphur-bearing constituent of the petroleum ether extract of wheat flour (preliminary report). *Cereal Chem.* 17: 243-245.
- Barton-Wright, E. C.
1938 Studies on the storage of wheaten flour. III. Changes in the flora and the fats and the influence of these changes on gluten character. *Cereal Chem.* 15: 521-541.
- Bull, Joan E.
1937 An investigation into the cause of the degrading effect of wheat germ on the baking quality of flour. *Cereal Chem.* 14: 244-256.
- Bungenberg de Jong, H. L.
1938 Proteins in the flour industry. Symposium on Proteins. Amsterdam.
- Eisenberg, S.
1940 Evidence in support of a recent paper concerning the effect of milk on the bromate requirements of flours. *Cereal Chem.* 17: 476-479.
- Flohil, J. T.
1936 The effect of chemical flour improvers on proteolytic action in relation to the gas-retaining capacity of fermenting doughs. *Cereal Chem.* 13: 675-689.
- Ford, W. P., and Maiden, A. M.
1938 Effects in dough of glutathione and papain. *J. Soc. Chem. Ind. (transactions)* 57: 278-281.
- Freilich, J.
1941 A method for the determination of reducing matter in flour. *Cereal Chem.* 18: 129-137.
- Freilich, J., and Frey, C. N.
1939 Dough oxidation and mixing studies. I. The action of potassium bromate in dough. *Cereal Chem.* 16: 485-494.
1939a Dough oxidation and mixing studies. II. Effects of remixing after fermentation. *Cereal Chem.* 16: 495-502.
1939b Dough oxidation and mixing studies. III. The effects of proteases and reducing substances on dough when mixed in oxygen. *Cereal Chem.* 16: 503-512.
1940 Dough oxidation and mixing studies. V. Correlation between reducing matter, protease activity and oxidizing effects in dough. Paper presented at A. A. C. C. meeting, New York, 1940 (manuscript seen).
1941 Dough oxidation and mixing studies. IV. Effects of oxygen and potassium bromate in sponge doughs. *Cereal Chem.* 18: 78-86.
- Geddes, W. F.
1930 The influence of germ constituents on baking quality and their relation to improvement in flour induced by heat and chemical improvers. *Can. J. Research* 2: 195-213.
- Geddes, W. F., and Larmour, R. K.
1933 Some aspects of the bromate baking test. *Cereal Chem.* 10: 30-69.
- Gould, I. A., Jr., and Sommer, H. H.
1939 Effect of heat on milk with especial reference to the cooked flavor. *Mich. Agr. Exp. Sta. Tech. Bul.* 164.
1939a Cooked and oxidized flavors of milk as affected by ferrous iron. *J. Dairy Sci.* 22: 1017-1023.

- Greenbank, G. R., Steinbarger, M. C., Deysher, E. F., and Holm, G. E.
1927 Effect of heat treatment of skim milk upon the baking qualities of the evaporated and dried products. *J. Dairy Sci.* 10: 335-342.
- Greenstein, J. P.
1938 Sulfhydryl groups in proteins. I. Egg albumin in solutions of urea, guanidine, and their derivatives. *J. Biol. Chem.* 125: 501-513.
1939 Sulfhydryl groups in proteins. II. Edestin, excelsin, and globulin in solutions of guanidine hydrochloride, urea and their derivatives. *J. Biol. Chem.* 128: 233-240.
1939a Sulfhydryl groups in proteins. III. The effect on egg albumin of various salts of guanidine. *J. Biol. Chem.* 130: 519-526.
- Grewe, Emily, and Holm, G. E.
1928 Effect of variation in the method of manufacture on the baking quality of dry skim milk. *Cereal Chem.* 5: 461-469.
- Hale, W. S.
1939 The proteinase in wheat flour. *Cereal Chem.* 16: 695-702.
- Hopkins, F. G.
1930 Denaturation of proteins by urea and related substances. *Nature* 126: 328-330.
1930a Denaturation of proteins by urea and related substances. *Nature* 126: 383-384.
- Johnson, A. H., and Ward, F. C.
1936 The relation between the viscosity of non-sweetened condensed skim-milk and baking quality. *Cereal Chem.* 13: 550-559.
- Jørgensen, Holger
1935 Ein Beitrag zur Beleuchtung der hemmenden Wirkung von Oxydationsmitteln auf proteolytische Enzymtätigkeit: Über die Natureder Einwirkung von Kaliumbromat und analogen Stoffen auf die Backfähigkeit des Weizenmehles. I. *Biochem. Z.* 280: 1-37 (also published in abbreviated form in *Muhlenlab.* 5: 113-126).
1935a Über die Natur der Einwirkung von Kaliumbromat und analogen Stoffen auf die Backfähigkeit des Weizenmehles. II. *Biochem. Z.* 283: 134-145.
1936 On the existence of powerful but latent proteolytic enzymes in wheat flour. *Cereal Chem.* 13: 346-355.
1938 Entwicklung der Theorie der Bromatwirkung, 1935-1938. *Muhlenlab.* 8: 201-208.
1939 Further investigations into the nature of the action of bromates and ascorbic acid on the baking strength of wheat flour. *Cereal Chem.* 16: 51-60.
- Kozmin, N. P.
1935 The aging of wheat flour and the nature of the process. *Cereal Chem.* 12: 165-171.
- Mirsky, A. E., and Anson, M. L.
1935 Sulfhydryl and disulfide groups of proteins. I. Methods of estimation. *J. Gen. Physiol.* 18: 307-323.
1936 Sulfhydryl and disulfide groups of proteins. II. The relation of number of SH and S-S groups and quantity of insoluble protein in denaturation and in reversal denaturation. *J. Gen. Physiol.* 19: 427-438.
- Munz, Emil, and Brabender, C. W.
1940 Extensograms as a basis of predicting baking quality and reaction to oxidizing agents. *Cereal Chem.* 17: 313-332.
- Ofelt, C. W., and Larmour, R. K.
1940 The effect of milk on the bromate requirements of flour. *Cereal Chem.* 17: 1-18.
- Read, J. W., and Haas, L. W.
1937 Baking quality of flour as affected by certain enzyme actions. III. Purified amylase and the relative proteolytic activity of amylolytic agents. *Cereal Chem.* 14: 58-73.
1937a Studies on the baking quality of flour as affected by certain enzyme actions. IV. Potassium bromate and enzyme activity. *Cereal Chem.* 14: 752-766.
1938 Studies on the baking quality of flour as affected by certain enzyme actions. V. Further studies concerning potassium bromate and enzyme activity. *Cereal Chem.* 15: 59-68.

- 1939 Studies on the baking quality of flour as affected by certain enzyme actions. VI. Further studies relating to the activation and inhibition of flour proteinase. *Cereal Chem.* 16: 60-70.
- Rich, C. E.
1934 The chemical and physico-chemical changes induced in wheat flour by artificial maturing agents. *Cereal Chem.* 11: 201-227.
- Samuel, L. W.
1934 The determination of amino-acids in wheat flour. *Biochem. J.* 28: 273.
- Skovholt, O., and Bailey, C. H.
1931 Relation of quality in dry skim milk to baking strength. *Cereal Chem.* 8: 374-380.
- 1932 The effect of temperature and of the inclusion of dry skim milk upon the properties of doughs as measured with the farinograph. *Cereal Chem.* 9: 523-530.
- Sullivan, B., and Howe, Marjorie
1937 The isolation of glutathione from wheat germ. *J. Am. Chem. Soc.* 59: 2742.
- Sullivan, B., Howe, Marjorie, and Schmalz, F. D.
1936 On the presence of glutathione in wheat germ. *Cereal Chem.* 13: 665-669.
- 1937 An explanation of the effect of heat treatment on wheat germ. *Cereal Chem.* 14: 489-495.
- Sullivan, B., Howe, Marjorie, Schmalz, F. D., and Astleford, G. R.
1940 The action of oxidizing and reducing agents on flour. *Cereal Chem.* 17: 507-528.
- Sullivan, B., and Near, Cleo
1933 Change of crude lipids of wheat on storage. *Ind. Eng. Chem.* 25: 100-103.
- Sullivan, B., Near, Cleo, and Foley, G. H.
1936 The role of lipids in relation to flour quality. *Cereal Chem.* 13: 318-331.
- 1936a The harmful effect of wheat germ on the baking quality of flour and the constituents responsible for this effect. *Cereal Chem.* 13: 453-462.
- Working, E. B.
1924 Lipoids, a factor influencing gluten quality. *Cereal Chem.* 1: 153-158.
- 1928 The action of phosphatides in bread dough. *Cereal Chem.* 5: 223-234.
- 1928a Some oxidizing effects of flour bleaching. *Cereal Chem.* 5: 431-435.
- Ziegler, E.
1940 Dough improvement studies. I. Oxidation of glutathione by potassium bromate. *Cereal Chem.* 17: 460-468.
- 1940a Dough improvement studies. II. The effect of adding oxidized glutathione to wheat flour doughs. *Cereal Chem.* 17: 551-555.
- 1940b Dough improvement studies. III. Further studies on the oxidation of glutathione by bromate, chlorate, and persulphate. *Cereal Chem.* 17: 556-564.
- 1941 Dough improvement studies. IV. Effect of some factors involved in respiration on the baking quality of wheat flour. *Cereal Chem.* 18: 167-185.

BOOK REVIEW

Advances in Colloid Science, Vol. I. By Elmer O. Kraemer, Editor, in collaboration with Floyd Bartell and S. S. Kistler. 434 pages. Interscience Publishers, Inc., 215 Fourth Avenue, New York. Price \$5.50.

This volume is the first of a series intended to provide organized and comprehensive surveys of recent discoveries in colloid science. The contributions are not merely reviews or compilations; the experimental and theoretical aspects of each development under consideration are presented by authors who by their own backgrounds are able critically to evaluate the existing data and techniques and to present the material in a uniform fashion.

The first volume includes a wide range of subjects, all of front-line importance and written for those directly engaged in the respective fields as well as for others whose interests may extend into these fields. For those interested in molecular and micellar structure, size, and shape, there is a chapter by Anderson on the developments to date with the newest tool for colloid studies, the electron microscope. Many photographs of various colloids are included. The implications of this work for other fields are stimulating. Also, there is a chapter by Edsall on the determination of size and structure from measurements of streaming birefringence. In another chapter by Powell and Eyring the basis for flow and the behavior of long-chain molecules are discussed on theoretical grounds.

Cereal chemists will welcome the 39-page chapter on the present status of starch chemistry by Kurt Meyer. He discusses the separation of starch into its components, the chemical constitution and structural characteristics of the components, and size distribution as determined by osmotic pressure and viscosity measurements. There is a section on the fine structure of starch grains and starch paste, and one on the enzymatic degradation of starch. The last part is on the constitution, degradation, and synthesis of glycogen.

Tiselius has a chapter on his new technique of absorption analysis, and shows by an example how the method can be used for studying protein hydrolysis.

Two chapters are included on the measurement of surface areas. Emmett discusses the method of low-temperature adsorption isotherms for finely divided or porous solids, and Sullivan and Hertel develop the theory and measurements for permeability methods applied to fibers and powders.

McBain deals with detergents from the point of view of their effects in solubilization. The new and interesting use of synthetic resins as agents for ionic exchange is surveyed by Robert Myers. Important work on the constitution of inorganic gels as determined by X-ray diffraction studies is described by Weiser and Milligan. The colloid chemistry of the creaming of rubber latex is ably discussed by Van Gils and Kraay.

Changes in surface tension of solutions of various substances with time and concentration are considered by Hauser in a chapter on anomalies in surface tension of solutions.

As a whole this book, the first volume of a long-awaited series, is highly satisfactory. It is only regretted that more space is not allowed for each subject, but this is partly counterbalanced by the excellent bibliography included with each contribution. The book is recommended to all interested in the techniques and interpretations of colloid chemistry and if the succeeding volumes are of equal merit, the series will be a most valuable contribution to colloid science.

HAROLD P. LUNDGREN, Western Regional Research Laboratory,
Bureau of Agricultural Chemistry and Engineering,
U. S. Department of Agriculture,
Albany, California

SUPPLEMENT

THE DETERMINATION OF MOISTURE IN THE WET MILLING INDUSTRY. I. CORN

L. SAIR¹ and W. R. FETZER²

The wet milling industry, composed of eleven refiners, process annually 80 to 100 million bushels of corn into starch, corn sirup, corn sugar, by-product feeds, and corn oil. In the wet milling process, the corn is first "steeped" in a dilute sulfurous acid solution, wherein the original moisture of the corn is increased to approximately 42% in order to soften the berry for resolution into the component parts of starch, protein, fiber, and germ. Starch is either dried as such or resuspended with water for acid hydrolysis into corn sirup or sugar. The germ is dried and expressed for its oil.

The entire process, as the name would indicate, is carried out through wet separation. All plants are "bottled up"; that is, water used in separation moves counter-current to corn in process. The water in this backward flow accumulates solubles, until it is finally treated with sulfur dioxide and used for soaking or steeping the incoming corn. This latter operation considerably increases the soluble content, and the steep water is finally removed from the system by evaporation and added to the various feeds.

In such separation, the question of losses becomes essential from the standpoint of operating efficiency. This efficiency, which is termed the "yield," is expressed as the quotient of the dry substance recovered by the dry corn substance introduced into the process. Normal losses will run between 2% and 3%. This loss has led to extended studies of losses through char used in refining, vacuum pan volatiles and entrainment, fermentation losses in processing, etc., which are fairly well known. Despite extended work in this direction, a large part of the loss was still unexplained. In terms of operation, a dry-substance loss of 2% to 3% was deemed impossible.

The entire yield picture is predicated on dry substance obtained from moisture data, and any discussion immediately reverts to whether or not the moisture data are accurate; that is, whether true moisture or relative moisture has been obtained. The controversy over moisture methods resulted in the Technical Advisory Committee of the Corn Industries Research Foundation initiating a study on the dry substance of corn sirup. This work resulted in data which showed

¹ Research Fellow, Corn Industries Research Foundation.

² Chief Chemist, Union Starch and Refining Co., Granite City, Illinois.

that the current methods for moisture in corn sirup underestimated the true moisture in a 43° Baumé sirup by as much as 1.4%. Since a considerable part of the output of every refiner is corn sirup, the data extended the loss in yield, and renewed the controversy. As a result, the Corn Industries Research Foundation established a full-time fellowship in the laboratory of a member company to investigate all moisture methods, in order to ascertain whether true moisture was being obtained with existing methods, and if not, whether true moisture could be obtained instead of relative moisture.

Although a large number of moisture studies have been conducted on cereals and feeds, their general findings have been far from satisfactory. Halvorson (1937) concluded that it is impossible to determine the true moisture content of cereals and feed products. Exhaustive studies on the moisture of wheat and flour by Snyder and Sullivan (1924, 1925, and 1926) led to the conclusion that the moisture method must be considered as a chemical tool which can yield only relative results. Nowak and Enders (1936) state that it is not possible to make an absolute determination of water in barley, malt, and hops, since the water is always present in different types of combinations. According to Nelson and Hulett (1920), water is held with great tenacity by cereals and appreciable amounts may still be present in materials heated to a high temperature. Gortner (1938) draws the conclusion from Nelson and Hulett's work that approximately 9% of the water in wheat flour is "bound" and that this water has no appreciable vapor pressure *in vacuo* at 100°C.

Kent-Jones (1939) in his recent book, *Modern Cereal Chemistry*, clearly expresses the present trend of thought on the subject of moisture in cereals: "The determination of the amount of moisture in flour is further complicated by the fact that portions of the water are apparently joined to the solid particles in varying degrees of affinity. To use a broad generalization mentioned earlier, a portion of the moisture would appear to be more 'moisture of constitution' than 'moisture of wetness.' When cereal products are heated, loss of moisture occurs. Initially, the loss is fairly rapid, but as the heating proceeds, and the moisture content becomes reduced, it becomes more and more difficult to remove further moisture. Apparently, some of the moisture in cereals is present in the free state (described above as 'moisture of wetness') while a portion of it is intimately associated or 'bound' with the proteins, etc. (described above as 'moisture of constitution'). The driving off of the 'bound' water by prolonged heating may quite alter the nature of the material. Generally, no sharp line of demarcation can be drawn between the two classes of moisture, and the determination of moisture, therefore, becomes an empirical process in which

the conditions must be carefully controlled if comparable results are to be obtained. Fortunately for the majority of purposes for which moisture determinations are conducted, absolute results are not necessary, and any process which yields comparable results may be made to serve."

The general consensus of opinion is that the true moisture content of cereals cannot be determined and that moisture methods must be looked upon as being purely empirical. As stated by Kent-Jones above, for the majority of purposes absolute moisture results do not appear to be necessary, and, therefore, empirical methods have been found to serve the purpose quite well. This is particularly true for the wheat milling industry, where the separation of the flour from the germ, shorts, and bran is purely physical. As a result, an empirical method for ground wheat, such as the vacuum oven method at 100°C, serves equally well for its various milled products. However, the situation in the corn milling industry is more complicated. Chemical as well as physical changes occur during milling. The various by-products differ markedly from each other in their properties. Obviously, an empirical method suitable for corn would be quite unsuitable for corn sirup or gluten feed. Each corn by-product requires the use of a moisture method suitable to its properties. As a result, it would follow that not one but at least three to five empirical moisture methods might be necessary for the estimation of moisture in corn and its products. These methods could be readily devised, and their use could lead to relative results. However, and this is the point which must be stressed, the results obtained would have little value in assessing the true efficiency in terms of yield of the milling process. Empirical moisture methods cannot yield the type of data required for the corn wet milling industry.

It was decided, therefore, to re-investigate the problem of moisture in corn and its by-products in order to ascertain whether some method could be devised whereby the determination of the true moisture could be accomplished. This paper is the first of a series dealing with moisture studies of the products, with the exception of sirups and sugar, common to the wet milling industry.

The moisture method for corn now accepted as official by the U. S. Department of Agriculture for grading purposes is the jacketed water-oven method. The method requires unground corn to be dried for 96 to 120 hours in a water oven heated to the temperature of boiling water (99°-100°C). Both the Brown-Duvel moisture tester (1907) and the Tag-Heppenstall moisture meter have been calibrated to give results in agreement with this method.

Considerable evidence has accumulated, suggesting that the water-oven method underestimates the moisture content. As early as 1889, Winton found that when corn meal was dried in a current of dry hydrogen for 5 hours at a temperature of boiling water, the results obtained were about 1% higher than those obtained by drying in air in an open dish. Hopkins (1898) also used the hydrogen drying method for corn, his conditions involving the drying of ground corn for 8 hours in hydrogen at 105°C. Recently, Cook, Hopkins, and Geddes (1934) have shown that the Brown-Duvel method consistently underestimates the moisture in wheat. Snyder and Sullivan (1924, Part I) found that flour yielded on the average 1.87% less moisture when dried in air as compared to *vacuo* at 100°C. Fabris (1931) stated that the Brown-Duvel method yielded results for corn which, on the average, were low by 0.73%. He recommended the use of a turpentine distillation method.

Recently Baehr (1940) found that the drying of corn at 100°C *in vacuo* resulted in an appreciable loss of volatiles. The amount of volatiles was determined, and by subtracting this value from the total weight loss a measure of the moisture present was obtained. He found that each of his drying methods yielded a different moisture value, and that constancy in weight was unobtainable since a slight loss continued over a long drying period. His results suggested that the determination of moisture in corn was quite empirical.

Plan of Investigation

The plan first involved a complete and thorough study of the moisture content of a single homogeneous sample of corn ground to pass a 40-mesh sieve. By so doing, both the grinding and sampling error could be largely eliminated. Moreover, by using finely ground material, it was believed that the moisture problem would be somewhat simplified, inasmuch as case-hardening, which hinders the liberation of moisture from within the particles, would be reduced to a minimum. Also, the rate of moisture removal would be greater than if corn of coarser mesh was used. Later, the plan involved a study of corn ground to a coarser mesh (10) and finally work was to be conducted with whole corn. This paper deals with the results obtained with the finely ground corn (40-mesh).

Five independent approaches were made to the moisture problem, which are summarized below:

- I. *Distillation Methods*: Direct water; high temperature; no vacuum.

Benzene.....	80°C	boiling point
Toluene.....	110°C	" "
Toluene-xylene		
(1-1 by volume)...	120°C	" "
Xylene.....	140°C	" "

- II. *Oven Methods:*
- a. Vacuum—indirect water; high temperature; high vacuum.
 - 70°C
 - 80°C
 - 100°C
 - 110°C
 - b. Air—indirect water; high temperature, no vacuum.
 - 100°C
 - 100°C over P_2O_5
- III. *De Bruyn:* Indirect water; low temperature; high vacuum.
 - 38°C
- a. Corn alone.
 - b. Corn soaked in 80% alcohol and dispersed on Hyflo Filter Aid.
- IV. *Extraction Methods:* Resolution into stable and unstable fraction with special treatment fitting each fraction.
- V. *Reversibility Studies:* Based on the assumption that water-holding capacity is a physical constant, if decomposition or chemical alteration has not occurred.

Analytical Methods

Materials: In order to secure a representative sample of corn, the following procedure was adopted. A quart of corn was secured from each of 14 cars of approximately the same moisture content, according to government grading. From this a composite sample of approximately 15 pounds was made. This was placed in a refrigerator and when cold was first ground through an attrition mill. That passing a 40-mesh screen was run into a 3-gallon bottle. The coarse portion was again placed in the refrigerator and when cold, reground in a coffee mill. The portion passing the 40-mesh screen was run into the bottle, and that retained was returned to the refrigerator. The operation was repeated until all of the sample passed 40-mesh. The combined 40-mesh sample in the bottle was shaken periodically over a period of 10 days to insure homogeneity of sample. The material was then transferred to 4-ounce screw-top, wax-lined bottles, and returned to the refrigerator until required for use.

Distillation methods: The distillation procedure followed was essentially that outlined by Bidwell and Sterling (1925), with the exception of specially designed apparatus. The distillation flask was a 250-ml Erlenmeyer with a 40/50 "standard taper" joint. The trap was of 5-ml capacity with 40/50 and 24/40 joints, each trap having been recalibrated at milliliter intervals.

The inner surfaces of traps and condenser were treated in the following manner to avoid entrained water globules: They were first scrubbed with cleansing powder. After washing with distilled water, they were allowed to stand full of freshly prepared cleaning solution for 4 hours. They were then washed with distilled water, followed by one washing with dilute caustic (1%) followed by distilled water, and then dried under room conditions for 24 hours.

The distillation rack was a modified extraction outfit built by the Precision Scientific Company to handle the above flasks, traps, and condensers. Six hot plates were available, each with vari-heat rheostat, which has been found invaluable in securing the proper rate of distillation for precise work. Each hot plate was equipped with a small stainless steel oil bath 4×4 inches, filled with corn oil. The oil bath has been found highly desirable in that heat is applied from the sides as well as the bottom, which results in more even boiling and lower temperature on the plate than when the flasks rest directly on the plate.

Twenty-five to 30 g of ground corn, depending on its moisture content, was weighed into a tared flask containing 5 to 8 g of asbestos, both of which had been previously dried in an air oven at 100°C . Asbestos has been found to prevent troublesome bumping, which causes erratic distillation. Sufficient solvent to cover the corn and asbestos (75 ml) was immediately added to the flask, which was then connected to the trap and condenser. After the trap was filled with solvent, distillation was started, and was continued until a period of 24 hours showed no increase in water.

Oven methods: The standard A.A.C.C. moisture dish was used (55×15 mm). The duplicate samples (4.5–5.5 g) include the sampling error, since the samples for single determinations were taken from different bottles of corn. The covers of the dishes were removed during drying but were quickly replaced upon removal of the dishes from the oven. Air passing through concentrated H_2SO_4 , Drierite, and P_2O_5 was used to release the vacuum in the ovens. Calcium carbide was used as the desiccant in the vacuum desiccator, 15 to 20 minutes being allowed for cooling of the samples.

Weber vacuum ovens were used. The pressure obtained ranged from 0.2 to 2.5 mm. The desired temperature was maintained within $\pm 1.0^{\circ}\text{C}$. No more than 6 to 10 samples were placed in the oven at one time. For air-oven tests, an Elconap oven was used.

De Bruyn method: De Bruyn and Van Laent (1894) devised an apparatus for the drying of maltose at low temperatures under vacuum in the presence of P_2O_5 . Cleland and Fetzer (1941) markedly improved the design of the apparatus, using No Lub standard taper joints throughout. It consists of two 250-ml pear-shaped flasks connected by a glass tube, $1\frac{1}{4}$ inches in diameter and 12 inches long. This apparatus is capable of retaining low vacuum (0.1 mm or better) for relatively long periods (24 to 72 hours) and has been found to be extremely satisfactory for drying corn and feeds at low temperatures under low pressure with P_2O_5 as a desiccant. The apparatus (Fig. 1) has a marked advantage over the customary vacuum desiccator

method, where the difficulty of maintaining the vacuum is always an important factor.

The corn (10–15 g) was weighed directly into a tared De Bruyn flask and the material was allowed to reach constant weight under high vacuum at a temperature of 38°C. After removal of the bulk of the water, the P_2O_5 was replaced with fresh desiccant.

Cleland and Fetzer had also shown that dispersion was an extremely important factor in the drying of sirups at low temperatures in the De Bruyn apparatus. They used Hyflo Filter Aid as a dispers-

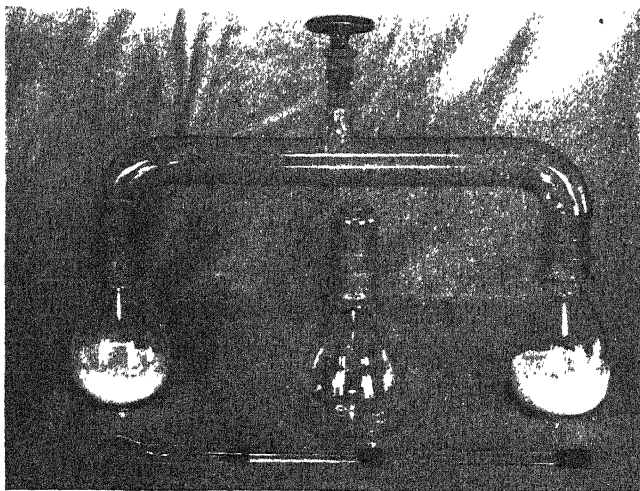


Fig 1. The De Bruyn apparatus

ing agent, and the sirup was intimately mixed with this material. It was thought that this technique might prove of value for corn.

As a result, the following procedure was also used: Ten to 15 g of corn was weighed into a weighing bottle and 50 to 75 ml of 80% alcohol by volume was added and the mixture allowed to stand overnight. It was then transferred to a tared de Bruyn flask containing approximately 20 g of vacuum dried (100°C) Filter Aid. The solvent was removed by placing the flasks in a vacuum oven at room temperature for 40 hours. It was thought that this procedure would result in the solution of the bulk of the corn protein, and as a consequence its finer dispersion on Filter Aid would result in a more ready loss of moisture.

The vacuums in the De Bruyn were determined daily and they were re-evacuated to 0.1 mm or less. Weighings were made at 24 to 72-hour intervals.

Extraction method: Eight to 10 g of corn was weighed into vacuum-dried (100°C) Whatman extraction thimbles contained in weighing bottles. A light plug of cotton wool was placed above the corn. The bulk of the moisture was removed from the corn by predrying the material for 48 hours at 80°C in a rapid stream of nitrogen. The corn was then extracted for 48 hours with ethyl ether. The ether-soluble fraction was taken to essential dryness on a steam bath, and was then dried to constancy in a stream of nitrogen at 75°C. The thimbles containing the ether-insoluble fraction were transferred to the weighing bottles and dried to constancy in vacuum at 100°C. By subtracting the weight of the ether extract from the total weight loss obtained in the thimbles, a measure of the moisture present in the corn was obtained.

Sampling and experimental error: In all cases, the difference between duplicates includes the sampling error, since the duplicate samples for each determination were taken from different bottles of corn. The check values for all determinations, with the exception of the oven results, are included in this paper. It will be noted that the distillation values agree within 0.10%, the De Bruyn values agree within 0.03%, and that the ether-extraction values agree within 0.06%. The SE of the duplicates from the means for the oven determination was 0.03%, the maximum difference between duplicates rarely exceeding 0.05%.

Moisture Results

Distillation results: Distillation methods have come into common use for the determination of moisture in a wide variety of products. The Brown-Duvel distillation method, based upon the principle and methods first described by Hoffman (1902), is now accepted as the official method for wheat, corn, and other cereals. The method is based on the principle of distilling the grain in a solvent immiscible with H₂O. The distilled water is collected and measured in some convenient form of measuring device. Dean and Stark (1920) were the first to develop the reflux type of apparatus, which was improved upon by Bidwell and Sterling (1925). The paper by Dean and Stark includes a comprehensive bibliography of all the work up to that date. Various immiscible solvents have been used which include commercial hydrocarbon products of varying boiling points—xylene, toluene—and recently use has been made of solvents of densities greater than water, such as carbon tetrachloride, tetrachlorethylene, and trichlorethylene.

Apparently no systematic attempt has been made to use the distillation methods for the determination of the true moisture present in cereals and feeds. The common practice has been to develop a distillation procedure against some other standard method, so that results are comparable. The Brown-Duvel, for instance, was standardized against the water-jacketed method. In their paper, Bidwell and Sterling stated that the distillation is usually complete within an hour when toluene is employed, and presented a table showing results for cereals and other substances in comparison with oven methods. Since distillation for an hour checked the oven method, it was deemed satisfactory and apparently no thought was given to whether added distillation time would give additional water or whether the oven methods represented true moisture. Thus (by an arbitrary procedure) a new technique on common products was tied to an oven method which, in turn, yielded relative moisture data.

Little if any account was taken of the fact that moisture might be retained more in one material than another and, therefore, would require additional distillation time or special experimental procedure. Thus for example the moisture data given by Bidwell and Sterling for Karo sirup is 20.3%. Fetzer and Evans (1935), using the same method but dispersing the corn sirup product on Filter Aid to make the moisture more available, obtained 27.5%. Thus, when a distillation method is standardized against another arbitrary procedure, it can only give moisture values of the same order of magnitude as the reference procedure, and may fail to indicate the true moisture content.

In this investigation a systematic use has been made of immiscible solvents of varying boiling points, carrying each distillation to completion. The moisture content of the 40-mesh corn was determined by distillation with benzene bp 80°C, toluene bp 110°C, a toluene-xylene mixture (1 : 1) bp 120°C, and xylene bp 140°C. The results obtained are shown graphically in Figure 2, and the final results are given in Table I.

It is of interest to note that the use of toluene, which boils at 30°C higher than benzene, yielded no further moisture. The solvents with higher boiling points gave a further moisture increase. However, it is well to point out that this additional increase was accompanied by the formation of an intense red color in the corn, indicating decomposition.

In the official toluene distillation method as given by the A.O.A.C. for moisture in feeds, which is essentially the method used in this investigation for the toluene procedure, it is stated that the entire process is usually over within one hour. This definitely has not been found true for corn and other feed-products used in our investigations. At the end of the three-hour period, the apparent moisture value of this

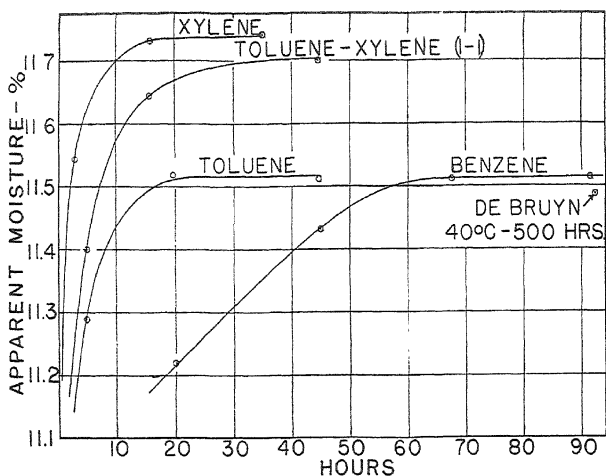


Fig. 2 Determination of moisture in corn by distillation methods.

TABLE I
DISTILLATION DATA FOR 40-MESH CORN

Solvent ¹	Length of distillation, showing period of constancy	Moisture determinations			Average
		hrs	%		%
Benzene	68-92	11.47, 11.49, 11.56			11.51
Toluene	20-45	11.49, 11.50, 11.54			11.51
Toluene-Xylene	16-45 ²	11.66, 11.74			11.70
Xylene	16-35	11.73, 11.74			11.74

¹ A correction factor of 0.02 ml is required for benzene and 0.03 ml for toluene. A factor of 0.03 ml was used for xylene and for the xylene-toluene mixture.

² Constancy not attained.

sample of corn by the toluene distillation method was still 0.4% too low, as compared with the final constant value. The distillation results will be discussed in a later section of this paper.

Oven results: The apparent moisture content of the corn was determined under various oven drying conditions. The corn was dried *in vacuo* at 70°, 80°, 100°, and 110°C. It was also dried in the air oven at 100°C over P₂O₅. The method used for drying the corn at 100°C over P₂O₅ was as follows: The moisture dishes were placed in the bottom of a 5-lb tin, and two glass containers containing P₂O₅ were supported above the corn samples. Two small holes were punched into the lid. Fresh P₂O₅ was used, as the conditions merited it. The complete data are shown graphically in Figure 3. The moisture value

obtained by the toluene distillation method is included in Figure 3, as well as the final value obtained by the De Bruyn procedure.

The results shown in Figure 3 clearly indicate that it is impossible to estimate the true moisture content of the corn by oven methods. Each time-temperature curve yields a different result. It might be thought that since the 100° and 110°C vacuum-oven results are in close agreement, the true moisture lies in this region. This assumption is completely erroneous, as will be shown later.

It is of interest to note that corn dried to constancy in an air oven at 100°C yields an apparent moisture value which is 1.14% lower than

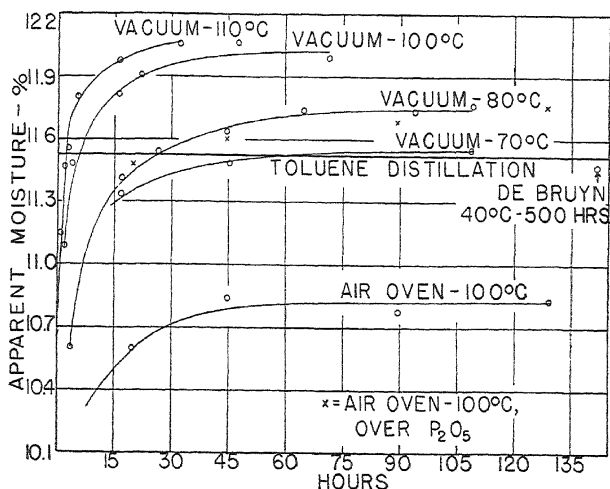


Fig. 3. Determination of moisture in corn by oven methods (40-mesh).

that obtained by drying the corn at 100°C *in vacuo*. That this difference is largely due to the ability of corn to retain water in an air oven at 100°C was proved by the fact that when the corn was dried in air over P_2O_5 at 100°C, this difference between air and vacuum drying largely disappeared, the difference being reduced to only 0.21%. If vacuum-dried corn is replaced in an air oven at 100°C, it actually adsorbs moisture, thus indicating the marked affinity of this material for water even at high temperatures (100°C). These results cast great doubt on the official water-jacketed-oven method, as a measure of true moisture.

The oven results will be referred to again in relation to the other data.

The De Bruyn method: The moisture content of the corn was determined by drying it over P_2O_5 under a high vacuum at a low temperature ($38^\circ C$). Other samples of corn were first "soaked" in alcohol and dispersed on Filter Aid before undergoing the same drying treatment. Both sets of De Bruyns, as shown in Table II, reached con-

TABLE II
THE DE BRUYN DRYING OF 40-MESH CORN—APPARENT MOISTURE

Length of drying	Corn		Corn +80% alcohol +Filter Aid	
	Duplicates	Average	Duplicates	Average
$38^\circ C$				
<i>hrs</i>	<i>%</i>		<i>%</i>	
275	11.32	11.33	11.31	11.30
600	11.45	11.50	11.47	11.45
700	11.45	11.51	11.49	11.45
800	11.45	11.49	11.46	11.45
$50^\circ C$				
An additional 100 hours	11.45 to 11.49		11.46 to 11.45	
	11.47		11.46	

stancy at the same moisture value, 11.46% to 11.47%. The results obtained indicated that the alcohol dispersion procedure resulted in no advantage over the untreated corn, either in the rate of moisture removal or in the final value obtained.

Within 275 hours of drying, the corn samples reached a moisture value within 0.20% of that obtained by benzene or toluene distillation. Constancy was reached within 600 hours, after which no further weight change occurred during an additional 200 hours of drying. Further drying for another 100 hours at $50^\circ C$ caused no additional moisture loss. The average constant value for the four determinations was 11.46%, which is within 0.05% of that obtained by distilling corn to constancy with benzene or toluene.

The connecting tubes of the De Bruyns were cleaned with cleaning solution prior to use, and if any volatiles had distilled over during drying, it should have been quite observable. Possibly a trace was present in two of the tubes, but the amount was unappreciable. The results show that corn can be dried at low temperatures ($38^\circ C$) to yield a moisture value essentially equal to that obtained by distilling corn to constancy with benzene or toluene. These results will be referred to again.

Ether extraction method: Baehr (1940) found that volatiles are given off from corn during vacuum drying at elevated temperatures. To correct for the volatiles, he quantitatively determined the amount given off and subtracted this value from the total weight loss during drying. The volatiles were trapped in a receiver surrounded by dry ice. The apparatus was necessarily somewhat elaborate, and in view of the small quantity of volatiles given off, a large sample of corn was required. As a result, the method is time-consuming.

A simpler method was chosen which would still account for the volatiles, but which would not require the use of flasks, connecting tubes, dry ice, large samples, etc. The method consisted in first extracting the volatiles from the corn and then drying the ether-soluble and ether-insoluble fractions separately to constancy by appropriate methods. Baehr had found that the volatiles were soluble in ether, and therefore this solvent was used for the extraction. The procedure used is given in the section under "Analytical Methods." The data are given in Table III.

TABLE III
ETHER EXTRACTION METHOD FOR THE DETERMINATION OF MOISTURE IN CORN

Ether-soluble fraction, % DS 75°C-stream of nitrogen			Ether-insoluble fraction, % DS 100°C <i>vacuo</i>			Moisture, %		
Hrs. of drying	Determinations (1) (2)		Hrs. of drying	Determinations (1) (2)		Determinations (1) (2)		Av.
	%	%		%	%	%	%	%
1	4.67	4.87	40	83.70	83.55	11.63	11.58	11.60
3	4.68	4.87	60	83.69	83.56	11.63	11.57	11.60

A second determination carried out by similar methods yielded a moisture content for the sample of corn of 11.56%. By removing the volatiles prior to drying, the apparent moisture content of the corn was reduced from 12.06% (vacuum drying at 100°C of corn direct) to 11.58%. This latter value is in fair agreement with the moisture results obtained by benzene or toluene distillation (0.07%) and with the De Bruyns (0.12%). Inasmuch as it is well recognized that ether is incapable of removing the total "fat" from corn, the slightly higher moisture results obtained by this procedure can, in all probability, be attributed to the small quantity of volatiles remaining in the ether-extracted material.

That volatiles are present in the ether extract of corn is readily apparent from Table IV. Upon completion of drying the ether extract to constancy in nitrogen at 75°C, the ether extracts were dried further in air at 100°C, followed by drying *in vacuo* at 100°C.

TABLE IV
THE EFFECT OF DRYING ON THE ETHER EXTRACT OF CORN

Drying method	Ether-soluble extract, % DS	
	Determinations	
	(1)	(2)
	%	%
75°C—N ₂ —3 hrs	4.68	4.87
100°C—air—2 hrs	4.56	4.76
4 hrs	4.66	4.85
26 hrs	4.52	4.62
100°C— <i>vacuo</i> —20 hrs	4.30	4.48

A weight loss first occurred in the air oven at 100°C, followed by a weight gain indicating oxidation of the extract. This was noted by Hopkins (1898). Continued drying of the extract at 100°C resulted in a considerable weight loss, which became very marked when the material was dried for an additional 20 hours *in vacuo* at 100°C. An over-all weight loss of 6.5% to 7.4% of the extract is indicated, and these values are probably low since a weight gain also occurred during the drying because of oxidation. If the vacuum-oven solids values of the ether extract are used in place of the values obtained at constancy in N₂ at 75°C, an apparent moisture value of 11.98% is obtained for the corn, which is a value corresponding closely to the results obtained by drying corn directly in the vacuum oven at 100°C.

The results obtained by this experiment clearly show that volatiles are given off during the drying of ground corn in vacuum at elevated temperatures. They also indicate that the marked variability in the oven data is attributable to the presence of volatile products.

Reversibility studies: The procedure for the determination of moisture in cereals, which has been termed the reversibility method, was first applied to gluten meal, and for this reason only the basis for the method will be given here together with the results obtained for corn. A fuller description covering its usage is given in a paper dealing with gluten meal.

It has been known for a long time that completely dehydrated biological products exhibit a hysteresis effect on transfer to their original storage condition. However, Urquhart and Eckersall (1930) and Pidgeon and Maass (1930) have shown that cellulose which contains 1% of moisture may lose it reversibly. In other words, let us suppose that cellulose is stored over H₂SO₄ and also over P₂O₅, and that under the former condition the cellulose still retains 1% of moisture, while under the second it is completely dried. According to the findings of the above investigators, the moisture that is lost on transfer of the cellulose from storage over H₂SO₄ to storage over P₂O₅ is completely regained on retransfer of the cellulose back to the H₂SO₄ storage

conditions. Obviously, if the cellulose were to undergo decomposition while being dried over P_2O_5 , this complete reversibility could not take place. These results offer the foundation for the reversibility method.

Relatively mild drying conditions are sufficient to remove all but the last 1% to 2% of moisture from cereal products. This last final increment is held with great tenacity as indicated by Table V.

TABLE V

WATER RETAINED BY THE PRODUCT WHEN IN EQUILIBRIUM WITH AIR AT 100°C¹

Product	Water retained
	%
Wheat flour	1.9 (Snyder and Sullivan, 1924, Part I)
Corn (40-mesh)	0.7
Corn (10-mesh)	1.0
Gluten meal (20-mesh)	0.5
Starch	0.9
Dextrin	0.4

¹ These values will fluctuate with the humidity.

In order to effect the removal of this final increment of moisture, it has become common practice to use vacuum ovens or higher-temperature air ovens. These methods, however, offer the possibility of dry-substance loss through volatilization or decomposition, and as a consequence it is always a problem to determine at just what point the total moisture is removed and also to determine whether this total loss is caused solely through moisture loss. An attempt was made to clarify this problem by utilizing the thought that the last increment of water may be removed reversibly.

The results obtained with cellulose were applied to the cereal moisture problem. A control sample of the cereal or feed was dried under conditions which eliminated any possibility of decomposition and which permitted the material to retain roughly 1% of moisture. Other samples were dried for various periods under drying conditions sufficient to remove the total moisture. These samples were then replaced in proximity to the controls, under which conditions the more completely desiccated samples readsorb moisture. Samples which adsorb sufficient moisture to yield a final apparent moisture value equal to the control are considered to be unchanged by the drying process. Those samples which are decomposed during the more drastic drying treatment, through the loss of volatiles or other causes, are unable to attain this similar equilibrium value.

There is an obvious fault in this reasoning which would lead to erroneous data with regard to this procedure—namely, the loss of volatiles or slight decomposition might alter the primary adsorptive power of the cereals. Normally, it would be necessary to prove or

disprove this possibility, but in view of the obtained data and the close relation of the moisture values obtained by this method to the other moisture methods used, it is believed that this possibility can be neglected.

In applying the reversibility method to a particular product, some experimentation is necessary to find the most suitable conditions. Since the method is based on the sorptive capacity of the cereal, it is essential that the material must be in a fine state of subdivision. Samples used for the moisture determination may be dried at any desired temperature and pressure by the usual oven procedures. Simultaneously, control samples are dried under conditions which permit the material to retain roughly 1% of moisture. In certain cases, when the product is stable, this can be accomplished by using the air oven at 100°C. In others, as with corn, the same conditions may be unsuitable since corn contains considerable fat, some of which is volatile and also readily oxidizable. The reversibility studies with corn were first carried out as follows:

The corn samples were dried for varying periods *in vacuo* at 100°C (Fig. 3), remoistened (8 ml H₂O to 4-5 g of corn), and the excess water was removed in the vacuum oven at room temperature. The control samples were given a similar moistening treatment. All samples were then carefully repulverized and allowed to reach complete equilibrium *in vacuo* at 40°C, which was largely attained within 200 hours and completely by 340 hours. The results given in Table VI are typical of

TABLE VI
REVERSIBILITY MOISTURE METHOD FOR CORN

Control	A Apparent moisture	B Reversible moisture value, 340 hrs at 40°C	C Adsorp- tion A-B	D Decom- position B-10.41%	E Calculated true moisture A-D
	%	%	%		%
Vacuum oven—40°C 340 hrs	10.41	10.41	—	—	—
Vacuum oven—100°C					
2 hrs	11.09	10.39	0.70	—	—
4 hrs	11.48	10.53	0.95	0.12	11.36 ¹
16 hrs	11.82	10.74	1.08	0.33	11.49
22 hrs	11.90	10.82	1.08	0.41	11.49
48 hrs	12.06	11.00	1.06	0.59	11.47
72 hrs	11.98	10.91	1.07	0.50	11.48
					Av. 11.48

¹ This value omitted from the average.

those obtained for the last three weighings (samples reweighed every 48 hours). The data are shown in a somewhat different form in Table VII.

TABLE VII
 DATA OF TABLE VI REARRANGED

Vacuum oven— 100°C	Loss in weight on transfer of corn dried to equilibrium in the vacuum oven at 40°C (10.41%) to the vacuum oven at 100°C	Regain in weight on replacement of 100°C vacuum oven samples to the vacuum oven at 40°C	Loss in recovery
hrs	%	%	%
2	0.68	0.70	+0.02
4	1.07	0.95	0.12
16	1.41	1.08	0.33
22	1.49	1.08	0.41
48	1.65	1.06	0.59
72	1.57	1.07	0.50

As shown in the tables, the two-hour drying period in the vacuum oven at 100°C resulted in no irreversible change as indicated by its complete recovery on retransfer to the vacuum at 40°C. Samples dried for longer periods, however, do not exhibit this complete reversibility. If complete reversibility is attained, the loss in weight should exactly balance the regain in weight, and where this is not attained, it might be attributed to a change in the adsorptive capacity of the heated samples or to the loss of volatiles or some other form of decomposition. It would be expected that with continued drying *in vacuo* at 100°C, a point would be reached where the total moisture is removed and additional heating results only in a dry-substance loss. If the adsorptive capacity of the dried products remained unchanged, they should be able to re-adsorb water to a degree dependent on their extent of drying. As shown above, those samples dried for 16 hours or longer at 100°C regain essentially the same weight; *i.e.*, they adsorb equivalent quantities of water. The constancy obtained, notwithstanding the fact that some of the samples were dried for an additional 56 hours, indicates that the adsorptive capacity was not affected. The data therefore show that the difference between the weight-gain and weight-loss values must be attributable to some irreversible loss during the vacuum drying in the oven at 100°C. The extent of this loss is shown in column D of Table VI, and when these values are subtracted from the apparent moisture results obtained in the vacuum oven at 100°C, it is found that the calculated true moisture values (column E) are in agreement for those samples dried for 16 hours or longer. Moreover, the results obtained by this reversibility method are in agreement with the moisture results obtained by the distillation methods, the De Bruyn procedure, and the ether extraction method.

A second reversibility study was conducted with several refinements. It had been found previously that an initial "wetting" is

essential in order to make all the samples comparable if vacuum-dried materials are required to adsorb considerable moisture. During the removal of the excess water, the possibility of changes, enzymatic or otherwise, is present. Work has shown that satisfactory results are obtainable without the need of first "rewetting" the material if the moisture differential between the apparent and reversible moisture values does not exceed 0.4% to 0.8%. In the second experiment, therefore, reversibility conditions were used which dispensed with this possible source of error. Since corn loses volatiles if the re-drying conditions are carried out *in vacuo* at more elevated temperatures (60°–80°C), the second study was carried out under atmospheric pressure. Work has shown that the use of an air oven at 60°–80°C is not satisfactory since humidity variations from day to day result in continued weight fluctuations. Moreover, the possibility of oxidation is present and the samples take up too much moisture. With these considerations in mind, the following conditions were used:

The corn samples dried *in vacuo* at 110°C were replaced in the Weber oven at 70°C. A constant stream of dry nitrogen was passed through the oven. By so doing, both humidity fluctuations and the possibility of oxidation were eliminated. Weighings were made from time to time and the results of typical weighings are given in Table VIII. It can be observed that the results agree admirably well with those obtained in the first study.

TABLE VIII
REVERSIBILITY MOISTURE METHOD FOR CORN

	A Apparent moisture	B Reversible moisture value	C Adsorp- tion A-B	D Decom- position B-11.14%	E Calculated true moisture A-D
<i>hrs</i>	%	%	%	%	%
Control ¹	11.14	11.14	—	—	—
Vacuum oven—110°C					
1½	11.46	11.22	0.24	0.08	—
2½	11.55	11.27	0.28	0.13	—
4	11.74	11.38	0.36	0.24	11.50
5	11.80	11.48	0.32	0.34	11.46
32	12.05	11.74	0.31	0.60	11.45
					Av. 11.47

¹ Samples dried for 96 hours at 70°C and for an additional 60 hours at 80°C in a stream of nitrogen.

Discussion

The moisture content of a single sample of corn ground to pass a 40-mesh sieve was determined by five different methods. Various modifications of each method were used. The complete data are summarized in Table IX, from which it can be observed that the ob-

TABLE IX
 SUMMARY OF MOISTURE DATA FOR 40-MESH CORN

Moisture method	Condition	Length of drying showing period of essential constancy	Final apparent moisture value
		<i>hrs</i>	<i>%</i>
Distillation	Benzene—bp 80°C	68–92	11.51
	Toluene—bp 110°C	20–45	11.51
	Xylene-Toluene—bp 120°C	16–45 ¹	11.70
	Xylene—bp 140°C	16–35	11.74
Oven drying	Vacuum—70°C	46–110 ¹	11.55
	80°C	65–110 ¹	11.77
	100°C	48–72 ¹	12.06
	110°C	16–32 ¹	12.05
	Air—100°C	45–130	10.84
	Air—P ₂ O ₅ —100°C	20–130 ¹	11.77
De Bruyn	Corn alone—38°C	650–850	11.48
	Corn alone—50°C	Additional 100	11.47
	Corn with alcohol—38°C	650–850	11.46
	Corn with alcohol—50°C	Additional 100	11.46
Extraction method	(1) Ether extract—75°C—N ₂	1–3	11.60
	Ether-insoluble—100°C— <i>vacuo</i>	18–36	
	(2) Ether extract—75°C—N ₂	2–6	
	Ether-insoluble—100°C— <i>vacuo</i>	24–48	11.56
Reversibility studies	(1) Corn predried—100°C— <i>vacuo</i>	—	11.48
	(2) Corn predried—110°C— <i>vacuo</i>	—	11.47

¹ Complete constancy not obtained.

tained moisture values range from 10.84% to 12.06%. The air-oven results (10.84%) are undoubtedly low, as shown by the fact that when the corn was dried over P₂O₅ in the air oven at 100°C, the moisture value obtained was 11.77%. Therefore the values deemed significant fall within the moisture range of 11.46% to 12.06%.

The results obtained by the ether extraction method clearly indicate that as much as 7% of the dry substance contained in the ether extract of corn is volatile *in vacuo* at 100°C. This percentage is equivalent to 0.45% of the original weight of the corn. By subtracting this percentage from the moisture values obtained by drying the whole corn *in vacuo* at 100° or 110°C, the obtained moisture range is reduced to include values within 11.46% to 11.61%. The moisture results obtained by the benzene or toluene distillation method, by the De Bruyn method of drying the corn under high vacuum at 38°C by the ether-extraction method, and by the reversibility method all fall within this relatively small moisture range.

The benzene and toluene distillation methods yielded identical results (11.51%) but immiscible solvents of higher boiling points caused a further increase in water obtained. This increase was

accompanied by an intense red color formation in the corn, which clearly suggests decomposition. By drying the corn to constancy *in vacuo* at 38°C over P_2O_5 , a moisture value of 11.46% was obtained. Further drying at an elevated temperature of 50°C resulted in no further moisture loss. The reversibility method, which has yielded very consistent data, indicated a calculated moisture value of 11.48%. These three diverse procedures all yielded results which fall within the very narrow moisture range of 11.46% to 11.51%.

The ether extraction method yields somewhat higher moisture values (11.56% to 11.60%). However, as pointed out previously, this method should tend to yield somewhat higher results since it is well known that ether does not extract the total "fatty" substances from corn. As a result the extracted material still contains a small percentage of volatiles, which may be expected to volatilize during the vacuum drying at 100°C to constancy. The final conclusion, which the results indicate clearly, is that the true moisture value for this sample of 40-mesh corn under test lies within the narrow range of 11.46% to 11.55%. The results of these experiments show that the true moisture content of corn can be determined.

This conclusion is at variance with that obtained by all previous investigators in the moisture field, who state that it is impossible to determine the true moisture content of cereals and feeds. The moisture method has been considered to be purely empirical. Much has been said with regard to "free" in contradistinction to "bound" water in cereals. Previously it was concluded that this "bound" water could not be differentiated from "water of constitution." A paper published by Nelson and Hulett (1920) has given strong support to the belief that water is held with great tenacity upon colloidal surfaces, and that appreciable amounts of water may still be present on materials heated to a high temperature. Gortner (1938) concluded from Nelson and Hulett's data that wheat flour contains approximately 9% of its total moisture in the "bound" form and that this water has no appreciable vapor pressure *in vacuo* at 100°C. Nelson and Hulett had shown that the apparent moisture content of a sample of flour dried at 100°C *in vacuo* was 10.80% and that the probable true moisture content was 11.80%, a difference of 1.0%. However, it must be pointed out that the flour was heated for only 4 hours at 100°C in a glass tube of narrow diameter, which led through a long capillary tubing to the receiver. Under ideal vacuum-oven drying conditions, it requires approximately 16 hours at 100°C to dry flour to constancy. With the restricted apparatus used by Nelson and Hulett, this period may have required lengthening to 100 hours. The obtained moisture value of 10.80% is not indicative of the true equilibrium value at

100°C and therefore the "bound" figure value calculated by Gortner for wheat flour is open to considerable doubt. From Nelson and Hulett's data, the present authors have no reason to believe that the calculated moisture value of 11.80% could not have been obtained if the flour had been heated for a sufficient length of time at 100°C. There is no reason to conclude from their results that temperatures in excess of 100°C are required to remove the moisture from cereals and feeds when dried *in vacuo*.

In the moisture study here reported, the conditions have been very diverse. Distillation solvents which varied by 30°C in boiling points yielded the same moisture result. The drying of corn over P_2O_5 at 38°C yielded a value essentially the same as that obtained by the use of an immiscible solvent boiling at 110°C. By drying the ether extract at 75°C in N_2 and the ether-insoluble fraction at 100°C in vacuum, results were obtained which were in good agreement with either the De Bruyn or distillation methods. The reversibility method, which appears to be based on sound premises, offers strong contributory data to that obtained by the other methods. The results clearly indicate that the moisture of corn can be differentiated from "water of constitution," and that it is unnecessary to rely on empirical methods for its estimation.

Summary and Conclusions

The moisture content of a sample of corn ground to pass a 40-mesh sieve was determined by five different methods, which included oven methods, distillation methods, drying under high vacuum over P_2O_5 , an ether extraction procedure by which the dry substances of the ether-soluble and ether-insoluble fractions were determined separately, and finally by a newly introduced procedure termed the "reversibility" method.

Distillation of corn with benzene or toluene yielded similar results (11.51%), while the use of solvents of higher boiling point resulted in higher moisture values. This increase was accompanied by an intense red color formation in the corn.

The oven methods yielded widely varying results, which ranged from the low of 10.84% for the air oven at 100°C (130 hours) to the high of 12.05% for the vacuum oven at 100°C (72 hours). It was demonstrated that the corn dried in the air oven still contained moisture, as shown by the fact that when the material was placed in the air oven at 100°C over P_2O_5 , a moisture value of 11.77% was obtained.

Corn can be dried to constancy at low temperatures (38°–50°C) *in vacuo* over P_2O_5 to yield a moisture value essentially equal to that obtained by the benzene or toluene distillation method (within 0.05%).

By first extracting corn with ethyl ether and then determining the dry substance in each fraction separately, a moisture value of 11.56% to 11.60% was obtained. These moisture values may be expected to be somewhat high since ether is generally considered to be unable to extract all the "fat" from corn. The ether extract of corn contains 6.5% to 7.4% of material which volatilizes *in vacuo* at 100°C. The results of this study unmistakably indicated that the major cause for the variability in the vacuum oven results can be attributed to the ether extract of corn.

The determination of the moisture content of the corn by the reversibility procedure yielded calculated moisture values ranging from 11.45% to 11.50%, which are in close agreement with the values obtained by benzene or toluene distillation (11.51%), by the De Bruyn method (11.46%), and by the ether extraction method (11.58%). The results of this investigation lend strong support to the reversibility method which is based on the assumption that during drying, no change occurs to the primary water-combining properties of the material.

The results of this study would clearly indicate that the *true* moisture content of corn can be determined.

Literature Cited

- Baehr, H. A.
1940 Progress reports on the moisture of corn. On file at Corn Industries Research Foundation, 5 East 45th Street, New York City.
- Bidwell, G. L., and Sterling, W. F.
1925 Preliminary notes on the direct determination of moisture. *Ind. Eng. Chem.* 17: 147-149.
- Brown, E., and Duvel, J. W. T.
1907 A quick method for the determination of moisture in grain. U. S. Dept. Agr. Bur. Plant Industry, Bul. No. 99.
- Cleland, J. E., and Fetzer, W. R.
1941-42 Determination of moisture in sugar products. *Ind. Eng. Chem. (Anal. Ed.)* 13: 858-860; 14: 27-30; 14: 124-129.
- Cook, W. H., Hopkins, J. W., and Geddes, W. F.
1934 Rapid determination of moisture in grain. *Can. J. Research* 11: 264-289.
- Dean, E. W., and Stark, D. D.
1920 A convenient method for the determination of water in petroleum and other organic emulsions. *J. Ind. Eng. Chem.* 12: 486-490.
- De Bruyn, Lobry C. A., and Van Laent, F. H.
1894 Maltose and its anhydride. *Rec. trav. chim.* 13: 218-222.
- Fetzer, W. R., and Evans, J. W.
1935 Baumé-purity-moisture tables for corn sirup. *Ind. Eng. Chem. (Anal. Ed.)* 7: 41-43.
- Fabris, U.
1931 Determination of moisture in corn. *Z. Untersuch. Lebensm.* 61: 345-347.
- Gortner, R. A.
1938 Outlines of biochemistry. John Wiley & Sons, Inc., New York.
- Halvorson, H. A.
1937 Report on moistures. *J. Assoc. Off. Agr. Chem.* 20: 435-438.
- Hoffman, J. F.
1902 Verfahren und Apparat zur Bestimmung des Wassergehaltes in Organischen Substanzen. *Z. angew. Chem.*, p. 1193, Berlin.
- Hopkins, C. G.
1898 The chemistry of the corn kernel. *Ill. Agr. Exp. Sta. Bul.* 53.

- Kent-Jones, D. W.
1939 Modern cereal chemistry. Northern Publishing Co., Ltd., Liverpool.
- Nelson, O. A., and Hulett, G. A.
1920 The moisture content of cereals. *Ind. Eng. Chem.* 12: 40-45.
- Nowak, G., and Enders, C.
1936 The determination of water in barley, malt, and hops. *Chem. Zentr.* 11: 202-203.
- Pidgeon, L. M., and Maass, O.
1930 The adsorption of water by wood. *J. Am. Chem. Soc.* 52: 1053-1069.
- Snyder, H., and Sullivan, B.
1924 Determination of moisture in wheat and flour, Part I. *Ind. Eng. Chem.* 16: 741-744. Part II, 16: 1163-1167.
1925 Determination of moisture in wheat and flour, Part III. *Ind. Eng. Chem.* 17: 311-314.
1926 Determination of moisture in wheat and flour, Part V. *Ind. Eng. Chem.* 18: 272-275.
- Urquhart, A. R., and Eckersall, N.
1930 The moisture relations of cotton. VII. A study of hysteresis. *J. Textile Inst.* 21: T499-T510.
- Wilbur, A. L.
1889 Conn. Agr. Exp. Sta. Report, p. 187.

THE DETERMINATION OF MOISTURE IN THE WET MILLING INDUSTRY. II. CORN

L. SAIR¹ and W. R. FETZER²

The results reported in Part I have proved that it is possible to determine the true moisture content of corn. Four diverse procedures, which clearly distinguished moisture from volatiles and decomposition, yielded moisture results which were in agreement.

This conclusion was based on the study of a single finely ground (40-mesh) homogeneous sample of corn. In practice, a coarser grind, such as a 20- or 10-mesh, would be much more desirable from the standpoint of preparation of sample. It was recognized that such a grind would produce small, hard grits from which moisture would be removed more slowly and perhaps not completely by some of the methods, such as the De Bruyn and benzene distillation, used successfully for the finely ground (40-mesh) material. Therefore it was decided to conduct a moisture study with a coarser grind—even greater than might be chosen for a laboratory method. For this purpose, a 10-mesh was chosen.

The first section of this paper is devoted to a moisture study of 10-mesh corn. The second section deals with the recommended official reference method for moisture in corn which was accepted by the Corn Industries Research Foundation (C. I. R. F.). The third shows the relation of the C. I. R. F. moisture method to the present official methods, and the final section shows the relation of the C. I.

¹ Research Fellow, Corn Industries Research Foundation.

² Chief Chemist, Union Starch and Refining Co., Granite City, Illinois.

R. F. moisture method to the methods now in common use by the members of the industry.

The Moisture of 10-Mesh Corn

For test purposes, a composite sample of corn was obtained by sampling ten cars of similar grading. Preparation of the sample and methods used followed the procedure described in Paper I.

TABLE I
DISTILLATION DATA FOR 10-MESH CORN—PERCENT MOISTURE

Benzene (200 hrs distillation)	Toluene (140 hrs distillation)
%	%
14.26	14.08
14.23	14.25
14.08	14.10
14.15	14.28
14.13	14.23
Av 14.17	Av 14.19

Distillation results: The moisture content of the 10-mesh corn was determined by distillation with benzene and toluene, and the results obtained are shown graphically in Figure 1, and the final values are shown in Table I.

The results indicate that both benzene and toluene yield essentially the same moisture value for 10-mesh corn. It will be observed that individual determinations for each method show considerably more variation than was obtained previously with 40-mesh material. This probably results from lack of homogeneity in the 10-mesh sample and failure to remove the last traces of moisture from the hard grits.

When 40-mesh corn was used, it was found that the benzene and toluene distillation methods reached constancy within 45 and 16 hours, respectively. With the 10-mesh corn, these periods were increased to 130 and 100 hours, respectively, a three- to six-fold increase. Granulation has a marked effect on the rate of water removal. This will be shown by experiments later in the paper.

The toluene distillation method is official for grain and stock feeds. The method states that the entire process is usually completed within an hour. At the end of two hours, the moisture value obtained for the sample of 10-mesh corn used above was 12.60%, a value which is still 1.59% lower than that obtained upon completion of the distillation. It would appear that an arbitrary assignment of an hour for distillation time can lead to erroneous results and the prerequisite time can be obtained only by a study of the specific material under investigation.

Oven results: The moisture content of the corn was determined by drying the material *in vacuo* at 80° and 100°C, and by the air-oven method at 100°C. The data are shown graphically in Figure 1.

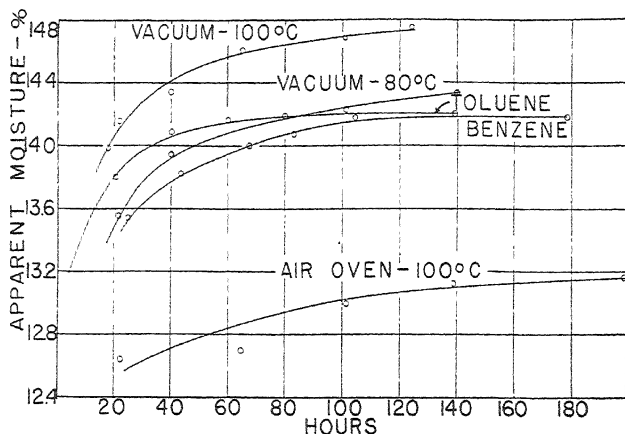


Fig. 1. Determination of moisture in corn by the oven and distillation methods (10-mesh).

The results obtained are substantially the same as those previously obtained with the finely ground corn, with the difference that the rate of moisture loss in all cases was markedly lessened. Whereas it required only 4 hours *in vacuo* at 100°C to dry corn (40-mesh) to a value equal to that obtained by toluene distillation, it required 22 hours for the 10-mesh corn.

The 100°C air-oven values were again markedly low as compared to the vacuum-oven results, a difference of 1.59% being obtained. In the previous study with 40-mesh corn, the difference obtained by the two methods was 1.22%. Apparently the coarser the granulation, the greater the differential. This is of importance since it is well to bear in mind that the standard official moisture method for corn involves the drying of whole corn in a water-jacketed air oven.

De Bruyn results: Ten-mesh corn was dried alone and in admixture with alcohol and Hyflo Filter Aid in De Bruyns at 50°C. The results in Table II show a considerable contrast to those previously obtained with the 40-mesh corn.

Within 275 hours at 38°C, the moisture values obtained with the 40-mesh corn were within 0.2% of those obtained by benzene or toluene distillation. Also, the alcohol treatment resulted in no advantage over the untreated corn. With 10-mesh corn at the end of 300 hours of drying at 50°C, the moisture values for the corn dried

TABLE II
THE DE BRUYN DRYING OF 10-MESH CORN—PERCENT MOISTURE

Drying Time, hr.	Corn			Corn + 80% alcohol + Filter Aid		
	Duplicates		Average	Duplicates		Average
	%	%	%	%	%	%
300	13.42	13.43	13.42	13.86	13.91	13.88
400	13.49	13.54	13.52	13.90	13.93	13.92
500	13.56	13.59	13.58	13.96	13.96	13.96
700	13.72	13.64	13.68	14.00	14.04	14.02
900	13.79	13.72	13.76	—	14.10	14.10

alone and for the alcohol-treated corn were 0.76% and 0.30% too low. Further drying up to 900 hours reduced this differential to 0.43% and 0.09%, respectively. The use of alcohol for the solution of the proteins and their dispersion in Filter Aid are very advantageous with coarsely ground corn. These results demonstrate again the important part played by granulation during the drying of materials at low temperatures. Although the untreated samples had not reached constancy even after prolonged drying, it appears doubtful whether sufficient moisture would be lost with further continued drying to equal the values obtained by the distillation methods. Rather it seems as though a constancy would be reached in which the corn would appear to be at equilibrium with the desiccant, but still containing entrapped water. As a result, a false equilibrium would be obtained which, if not recognized, would lead to false conclusions. It might be concluded that the lower moisture values are due to "bound" water or to the water-binding capacity of the corn, rather than to the true reason, which is "case-hardening" or the unavailability of the water to the desiccant.

After 900 hours of drying, the alcohol-treated corn yielded a value within 0.09% of the toluene distillation. Inasmuch as constancy was not reached, these results add further confirmation to the belief that corn can be dried *in vacuo* at low temperatures over P_2O_5 to yield values essentially similar to those obtained by distillation.

Ether extraction method: The moisture content of the 10-mesh corn was determined by the ether extraction method, as previously described. The results are given in Table III.

The ether extraction method yields a calculated moisture content of 14.28%, in contrast to the value of 14.75% obtained by drying the whole corn directly *in vacuo* at 100°C. These results again demonstrate the error involved by vacuum drying of corn at 100° C through the loss of volatiles. The ether extraction value (14.28%) is reasonably close to that obtained by toluene distillation (0.0% %).

TABLE III
EXTRACTION METHOD FOR THE DETERMINATION OF MOISTURE IN 10-MESH CORN

Ether-soluble fraction, C_e DS 75°C—stream of nitrogen			Loss in weight of the ether- insoluble fraction—100°C— <i>vacuo</i>			Moisture		
Hrs. of drying	Determinations		Hrs. of drying	Determinations		Determinations		Av.
	(1)	(2)		(1)	(2)	(1)	(2)	
2.5	C_e 3.90	C_e 3.83	40	C_e 18.17	C_e 18.09	C_e 14.27	C_e 14.26	C_e 14.26
3.5	3.90	3.83	64	18.18	18.11	14.28	14.28	14.28

As found previously for 40-mesh corn, this method tends to yield somewhat higher results, which it is believed can be attributed to the presence of traces of volatiles remaining in the extracted corn.

Reversibility: Considerable difficulty was experienced in the application of the reversibility method to coarsely ground corn (10-mesh). As shown in the section on De Bruyn methods, coarsely ground corn tends to reach a false equilibrium when dried under mild conditions, because of the unavailability of the "entrapped" water to the desiccant. As a result, the De Bruyn method on the untreated sample underestimates the moisture content.

It has been pointed out previously that reversibility experiments must be conducted under drying conditions which eliminate any possibility of decomposition. Otherwise the check samples of corn would undergo some change. However, if the check samples of 10-mesh corn are dried under the conditions essential for a proper reversibility study, we then find that the material behaves in a manner similar to that exhibited by the corn in the De Bruyns. An equilibrium condition is reached with continued drying but the moisture value obtained, if accepted, would lead to false conclusions. The factor of granulation is an inhibiting influence, as the moisture value is much lower than would normally be obtained. As a result, the check moisture value cannot be used as a basis of comparison against the previously heat-treated samples used for moisture determinations. It has therefore been found impossible to draw any conclusions from the studies conducted with coarsely ground corn without first making an added assumption.

The reversibility studies with 40-mesh corn were carried out without encountering the difficulties mentioned above. Three different studies were conducted, and in each case it was found that the ratio of water to volatile loss (decomposition) was essentially the same; i.e., it was found that when the apparent moisture value of the corn equaled the toluene distillation value, decomposition equivalent

to 0.1% had occurred. We have therefore assumed that this same ratio also holds for 10-mesh corn. Observation of the data in Table IV shows that the drying of 10-mesh corn for 16 hours *in vacuo* at 100°C resulted in an apparent moisture value of 14.00%, a value within 0.19% of that obtained by toluene distillation. In view of the work carried out with 40-mesh corn, it has therefore been assumed that a volatile loss equal to 0.1% had occurred during this drying period.

Corn samples dried for varying intervals *in vacuo* at 100°C were re-dried in a stream of nitrogen at 85°C. Drying was continued for 168 hours, and essentially the same result was obtained for the final three weighings. Typical results obtained after 96 hours of drying are given in Table IV.

TABLE IV
REVERSIBILITY OF 10-MESH CORN

Sample	A Apparent moisture	B Reversible moisture	C Adsorption A-B	D Decompo- sition B-13.98%	E Calculated true moisture A-D
	$\%$	$\%$	$\%$	$\%$	$\%$
Check	13.76	13.76 ¹	—	—	—
Calculated check	13.98	13.98	—	—	—
Vacuum oven, hrs					
16	14.00	14.08	—	0.10 ²	—
40	14.40	14.24	0.16	0.26	—
64	14.58	14.34	0.24	0.36	14.22
88	14.65	14.38	0.27	0.40	14.25
136	14.65	14.41	0.24	0.43	14.22
					Av 14.23

¹ Check value too low—false equilibrium.

² Decomposition equal to 0.1% was assumed in the light of studies carried out with 40-mesh corn.

It will be observed that the moisture value obtained by the reversibility method is in close agreement with the moisture results obtained by the distillation and ether extraction methods. These results will again be referred to in relation to the other data. A second reversibility study was conducted in which the samples were all "wetted" with 50% alcohol prior to drying under the reversibility conditions, but nevertheless it was still found that the check sample yielded low results. Using the assumption made above, it was found that the average calculated moisture value for this second study was 14.18%.

Discussion of the results obtained with 10-mesh corn: The complete data obtained with 10-mesh corn is summarized in Table V. As can be observed from the data given in Table V, the moisture values obtained with the 10-mesh corn range from 13.16% to 14.75%. Values below 14.17% can undoubtedly be rejected as being too

TABLE V
SUMMARY OF MOISTURE DATA FOR 10-MESH CORN

Moisture method	Drying conditions	Length of drying <i>h, s</i>	Final moisture value <i>%</i>
Distillation	Benzene—BP 80°C	200	14.17
	Toluene—BP 110°C	140	14.19
Oven drying	Vacuum—80°C	140 ¹	14.33
	100°C	125 ¹	14.75
	Air—100°C	200 ¹	13.16
De Bruyn	Corn alone—50°C	900 ¹	13.76
	Corn with alcohol—50°C	900 ¹	14.10
Extraction method	Ether extract—75°C—N ₂	4.5	—
	Ether-insoluble—100°C— <i>vacuo</i>	64	14.28
Reversibility study	Corn predried—100°C— <i>vacuo</i>	(1)	14.23
		(2)	14.18

¹ Constants not obtained

since the studies conducted with 40-mesh corn clearly indicate that the benzene distillation method causes no decomposition in corn. Similarly, values above 14.28% can be dismissed, since the results by the ether extraction method clearly show that the higher values include the loss of volatiles. The significant moisture range therefore includes values between 14.17% and 14.28%. Three methods have yielded results which fall within this narrow moisture range: the distillation methods (benzene 14.17%, toluene 14.19%), the extraction method (14.28%), and the reversibility method (14.23%, 14.18%).

The results obtained with 10-mesh corn are essentially in agreement with those obtained with 40-mesh corn. The differences noted can be clearly attributed to the effect of granulation. The results demonstrate that the total moisture can be removed from the coarsely ground material if the drying period is sufficiently lengthened to compensate for the inhibiting effect of the coarser granules on moisture loss, and further, that extended times necessary for such moisture removal are justified since these periods produced no decomposition, volatiles, etc. with 40-mesh corn.

The C. I. R. F. Official Reference Moisture Method for Corn

In the studies which have been conducted on 40- and 10-mesh corn, the following methods, both direct and indirect, have narrowed the true moisture content to very narrow limits: (1) distillation with benzene and toluene, (2) De Bruyn, (3) resolution by ether into two fractions, and (4) reversibility.

The distillation procedures stand out as they yield direct moisture and are more adaptable to general laboratory routine. Both benzene and toluene distillation have yielded essentially similar values. Inasmuch as the toluene method is not nearly as time-consuming, the C. I. R. F. adopted the toluene distillation method and a granulation of 20-mesh corn as the official method for the determination of moisture in corn.

The problem of proper sampling and grinding of whole corn in the two studies on 40-mesh and 10-mesh corn was purposely avoided by using, in both cases, a single well-mixed material which, in the case of 40-mesh, can be regarded as homogeneous, and in the case of the 10-mesh, substantially so. To translate these results to laboratory practice requires that the two important factors of sampling and grinding be taken into account. Both can introduce serious error in evaluating the moisture content of corn, independently of the final moisture method used.

As to grinding, it is well known that regardless of the method used, some moisture is lost, particularly if the moisture is high. In the double weighing method, wherein the whole grain is allowed to reach equilibrium in a conditioned room prior to grinding, and the change in weight computed to the final value, there still occurs some moisture loss in grinding.

In order to reduce the grinding loss to a minimum, the C. I. R. F. method proposes that the small Wiley mill be used (No. 4267-P Intermediate Model). This mill permits the quantitative recovery of the sample, and hence the moisture loss during grinding can be determined. It has been found very satisfactory for reduction of whole corn to 20-mesh, with grinding losses which are negligible.

The C. I. R. F. official reference moisture method for corn is as follows: Twenty to 30 g of corn, depending upon the moisture content, is weighed into a tared weighing bottle. The sample is then ground quantitatively in the Wiley mill to 20-mesh and transferred into the weighing bottle, and the moisture loss during grinding is determined. The ground sample is then transferred to a previously air-dried (100°C) distillation flask (250 ml) containing a mat of asbestos, and distillation continued until no water is given off (20 to 80 hours). A more detailed procedure covering apparatus, preparation of the sample and other details is given in the final section following these papers.

Relation of the C. I. R. F. Moisture Method to the Official Methods Now in Common Usage

Having adopted the reference method for the determination of moisture in corn, it seemed desirable to ascertain how the value

obtained compared to those obtained by state grading laboratories and those within the industry. Such a comparison entailed the preparation of a large quantity of corn of uniform moisture content.

So much has been written regarding the necessity of proper sampling that it is unnecessary to stress again the importance of this factor in moisture studies. Four lots of different grading and moisture were desired. The procedure adopted was as follows: Each lot of corn was allowed to reach equilibrium by being sealed in a metal can for three weeks. It was then transferred to a four-gallon bottle and vigorously shaken for several hours, transferred to one-pound friction-top tins, and stored in a refrigerator. It is believed that this procedure for handling small lots of test samples will lead to more uniformity in whole grain than can be obtained by the use of a Boerner sampler.

This comparative study among laboratories is open to criticism in that it is based on only four samples. However, the results obtained have been in agreement and the data appear to be conclusive. Pound samples were forwarded to (1) two state grain laboratories, (2) eight laboratories within the industry, and (3) samples were retained for moisture tests.

The request to the state grain laboratories was that the moisture be determined by the official method which, in both cases, was the Tag-Heppenstall method. The member laboratories were asked to determine the moisture by methods which they considered most accurate. The samples retained were run by the official C. I. R. F. method and by the official reference method of the U. S. D. A. (100°C electric oven instead of water-jacketed air oven).

The four samples had been graded by the local Merchants Exchange as follows:

Sample No.	Grade	Moisture
1	No. 1 Yellow	11.60%
2	No. 2 Yellow	12.90%
3	No. 2 Yellow	14.40%
4	No. 3 Yellow	16.40%

The moisture contents of the four samples of corn were determined by the C. I. R. F. method with the results shown in Table VI.

In a second study, benzene was used in place of toluene and it was found, as shown in Table VII, that the two distillation methods agree reasonably well. These results also show that the sampling error between the one-pound tins was very small.

The results in Tables VIII, IX, and X show a comparison of the C. I. R. F. method with the now accepted official method of the U. S. D. A. Unfortunately the samples sent to the two government laboratories were considered somewhat small, but those who made

TABLE VI
MOISTURE OF CORN BY THE C. I. R. F. METHOD

Sample No.	Determination	Moisture loss during grinding	Moisture—toluene (60 hrs distillation)	Corrected moisture value for grinding loss
		%	%	%
1	1	0.38	13.31	13.69
	2	0.37	13.24	13.61
				Av 13.65
2	1	0.30	13.92	14.22
	2	0.29	13.86	14.15
				Av 14.18
3	1	0.44	15.76	16.20
	2	0.45	15.63	16.08
				Av 16.14
4	1	0.57	17.00	17.57
	2	0.56	17.03	17.59
				Av 17.58

TABLE VII
MOISTURE OF SAMPLE NO. 4 BY BENZENE AND TOLUENE—PERCENT MOISTURE

Benzene (124 hrs distillation)	Toluene
%	%
17.45	17.57
17.50	17.59
Av 17.48	Av 17.58

TABLE VIII
THE C. I. R. F. MOISTURE METHOD FOR CORN VS U. S. D. A. METHOD

Sample No.	C. I. R. F. moisture value	U. S. D. A. 100°C air-oven method (170 hrs) whole corn	U. S. D. A. Tag-Heppenstall values			
			Official moisture value for purchased car	State grain laboratory	Merchants exchange laboratory	Average Tag-Heppenstall value
1	13.65	11.75	11.60	11.30	11.03	11.31
		11.68 Av 11.72				
2	14.18	12.31	12.90	12.90	12.30	12.70
		12.26 Av 12.28				
3	16.14	14.20	14.40	14.50	14.80	14.56
		14.27 Av 14.24				
4	17.58	15.66	16.40	—	16.45	16.42
		15.64 Av 15.65				

the tests stated that the results obtained represented fairly well the moisture present. The moisture determinations were carried out by the Tag-Heppenstall electric moisture method, which has largely superseded the Brown-Duvel method. The Tag-Heppenstall method

TABLE IX
C. I. R. F. vs TAG-HEPPENSTALL

Sample	C. I. R. F. method	Tag-Heppenstall	Difference
	%	%	%
1	13.65	11.31	2.34
2	14.18	12.70	1.48
3	16.14	14.56	1.58
4	17.58	16.42	1.16

TABLE X
C. I. R. F. vs AIR-OVEN—100°C

Sample	C. I. R. F. method	Air-oven—100°C (170 hrs)	Difference
	%	%	%
1	13.65	11.72	1.93
2	14.18	12.28	1.90
3	16.14	14.24	1.90
4	17.58	15.65	1.93

was calibrated, as was the Brown-Duvel, against the water-jacketed oven method.

It will be noted that the Tag-Heppenstall method underestimates the moisture in corn by values ranging from 1.16% to 2.34%. Presumably the lower the moisture of the corn, the greater the difference. Since the Tag-Heppenstall was calibrated against the water-jacketed oven the results given in Table X are even more significant. It was found that the air-oven method at 100°C underestimates the moisture in corn by 1.90% to 1.93%. Since it is generally recognized that the water-jacketed oven method yields lower results than the air-oven method at 100°C, it seems safe, therefore, to conclude that the present official U. S. D. A. method for corn underestimates the moisture content of corn by at least 2.0%.

The results shown in Tables XI and XII clearly indicate that whole corn retains a portion of its moisture with great tenacity.

TABLE XI
C. I. R. F. vs VACUUM OVEN—100°C

Sample	C. I. R. F. method	Vacuum oven 100°C (150 hrs)	Difference
	%	%	%
1	13.65	13.00	0.65
2	14.18	13.54	0.64
3	16.14	15.30	0.84
4	17.58	17.08	0.50

U. S. D. A. lab

TABLE XII
C. I. R. F. vs AIR-OVEN—130°C

Sample	C. I. R. F. method	Air-oven 130°C (50 hrs)	Difference
	%	%	%
1	13.65	12.74	0.91
2	14.18	13.30	0.88
3	16.14	15.11	1.03
4	17.58	16.68	0.90

Samples of whole corn (10 g) were dried *in vacuo* at 100°C and in air at 130°C for a lengthy period and, as can be observed, a drying period as long as 150 hours *in vacuo* at 100°C is still insufficient for the total removal of the moisture from whole corn. These results show the inadequacy of the present water-jacketed-oven method for the estimation of moisture in whole corn.

Whereas finely ground corn loses its moisture with readiness, whole corn retains a portion of it with remarkable tenacity. During this investigation it has been found that at 100°C *in vacuo*, the total moisture in 40-mesh corn is removable within 4 hours, in 10-mesh corn it required 40 hours, and apparently whole corn requires at least 200 to 300 hours. Granulation plays an important part in rate of water removal. This is again clearly demonstrated in Table XIII.

TABLE XIII
C. I. R. F. vs COMPANY NO. 7 METHOD

Granulation	Company No. 7 method	C I R. F. method	Difference
			%
40-mesh	11.56	11.51	+0.05
10-mesh	13.77	14.19	-0.42
1/4 grain	12.53	14.18	-1.65

TABLE XIV
MOISTURE METHOD OF COMPANY NO. 7 vs U. S. D. A. METHOD

Month, 1941	Company No. 7 (monthly average)	State grade moisture (monthly average)	Difference
	%	%	%
January	18.15	17.70	0.45
February	18.18	17.92	0.26
March	17.57	17.37	0.20
April	16.42	15.99	0.43
May	14.69	14.14	0.55
June	13.96	13.63	0.33
			Av 0.36

The method of Company No. 7 is an empirical routine procedure standardized against the water-oven method. It is used as a check against car moisture grading and for factory dry substance purposes. It consists of heating 25 g of $\frac{1}{4}$ grain corn in a vacuum oven ($28\frac{1}{2}$ -inch vacuum) at 115°C for 5 hours. The method, in use for years, usually checks car grading and is currently giving values shown in Table XIV.

Relation of the C. I. R. F. Moisture Method to the Methods Now in Use in the Corn Products Industry

As stated in Paper No. I, the corn products industry annually purchases upwards of 100,000,000 bushels of corn, or approximately one-third of the cash corn. This work on moisture in corn has been sponsored by the Corn Industries Research Foundation, because many laboratories of the members believed that official methods do not represent true moisture. Some laboratories have procedures which they believe give more accurate data, while some have adhered to routine methods based on U. S. D. A. official methods. Only one company employs the Brown-Duvel method (No. 8). The others employ oven methods. A comparison of the industry's methods with the C. I. R. F. method is shown in Figure 2 and given in Table XV.

It is of interest to note that the Brown-Duvel method (No. 8) underestimates the moisture in corn samples by values ranging from

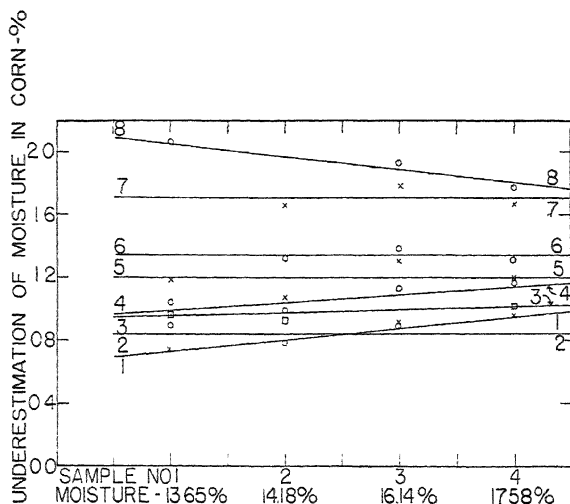


Fig. 2. Relation of the Corn Industries Research Foundation reference method to methods used in the corn milling industry. The numbers refer to companies (see text).

TABLE XV
C. I. R. F. vs INDUSTRY METHODS

Company	Underestimation of moisture				Average underestimation of companies
	Sample No				
	1	2	3	4	
	%	%	%	%	%
1	0.74	0.78	0.92	0.97	0.85
2	0.90	0.79	0.90	—	0.86
3	0.98	0.92	—	1.03	0.98
4	1.05	0.99	1.14	1.17	1.09
5	1.18	1.07	1.31	1.22	1.20
6	—	1.32	1.39	1.31	1.34
7	—	1.65	1.79	1.66	1.70
8	2.06	1.38	1.94	1.78	1.79

1.38% to 2.06%, the average difference being 1.79%. These results are in essential agreement with those found previously where the Tag-Heppenstall values were on the average too low by 1.75%, and the air-oven underestimated the moisture by 1.92%.

It will be noted in Table XIV that the method of Company No. 7, over a six-month period, yielded an average moisture value which was 0.36% higher than that obtained by the State Grain Tag-Heppenstall method. This company, as shown in Table XV, underestimated the moisture in corn as compared to the C. I. R. F. method by 1.70%. Indirectly, therefore, we obtain added confirmation indicating that the now accepted official moisture method for corn is, on the average, low by approximately 2.0%.

Conclusions and Summary

The Corn Industries Research Foundation has presented a reference method for the determination of the true moisture in corn. A comparison of this method with the prevailing official methods indicates that:

1. The official water-jacketed-oven method underestimates the moisture in corn by approximately 2.0%, which is a constant difference over the moisture range of 12%-18%.
2. The official Brown-Duvel method underestimates the moisture in corn by values ranging from 1.4% to 2.1%.
3. The official Tag-Heppenstall meter underestimates the moisture in corn by values ranging from 1.2% to 2.3%.
4. The various methods in use by the corn products industry designed to obtain more accurate moisture data, still underestimate the moisture by 0.8% to 1.8%.

THE DETERMINATION OF MOISTURE IN THE WET MILLING INDUSTRY. III. THE MILL PRODUCTS

L. SAIR¹ and W. R. FETZER²

The first two papers in this series covered the determination of moisture in corn. Subsequent papers deal with the determination of moisture in the by-product feeds—gluten meal, corn oil meal, and gluten feed. Inasmuch as chemical as well as physical changes occur during wet milling, which may profoundly alter the nature of the resulting feeds, particularly with respect to their moisture determination, a specific study of each feed product was considered desirable. While standard moisture methods can be applied to a large number of allied products, whether or not true moisture is obtained for the specific product must be determined by a special moisture study of that product. The common assumption that a moisture method can be applied at random is basically one of the major reasons why moisture values have been termed empirical.

The three by-product feeds produced by the wet milling industry are corn gluten feed, corn gluten meal, and corn oil meal. Although the sales specifications among companies may differ to some extent, the following guaranteed analysis may be considered typical:

	Gluten meal	Gluten feed	Corn oil meal
	%	%	%
Crude protein, not less than	41	25	20
Crude fat, not less than	1	2	8
Crude fiber, not more than	5	8.5	10
NFE, not less than	43	43	—

Two of the above feeds, gluten feed and gluten meal, are blends of end products in the milling process. Corn oil meal is simply ground expeller cake. Gluten feed is a blend of coarse hull or bran, steep water, and sufficient corn gluten to make minimum protein guarantees. Gluten meal is a blend of corn gluten, fine fiber or No. 17 tailings, and, at times, some steep water.

The determination of moisture is carried out in the factory not only on the components which make up the feeds but also on the finished feeds. The moisture methods on the latter become important inasmuch as this determination is also made by outside laboratories, making common methods essential. For this purpose relative methods would suffice, but for the purpose of computing yields true moisture values are imperative.

A study of the feed components, which are the hulls, corn gluten, steep water, was considered desirable in the approach to the

Fig. 2. ¹Research Fellow, Corn Industries Research Foundation
²Chief Chemist, Union Starch and Refining Co., Granite City, Illinois.

problem of true moisture of feed by-products, since this investigation would yield an insight into the behavior of the finished feeds. As a result this paper is concerned with a moisture study of the constituents which are used in the manufacture of the finished feedstuffs. It is of importance to note, however, that the behavior of the feeds cannot be altogether predicted from a study of their constituent parts since there is an important point of difference. The component parts carry with them to the driers water containing corn solubles and miscellaneous products of fermentation and enzymic action. During drying, some of these products are lost while others may have reacted to form more stable products. Severe case-hardening, with the resultant entrapment of water, may also occur. As a result the finished feeds may be expected to differ in some respects, both chemically and physically. Before discussing the moisture work on the components, a brief summary of how they originate in the process is in order.

In the wet milling process the water used for washing the final starch moves countercurrently to the corn through a series of separators, in which starch is washed free. The water accumulates solubles and other products of fermentation and enzymic action during this course until it meets the incoming corn. At this stage SO_2 is added to the water in the magnitude of 0.2% to 0.3% and to this the name of steep acid is applied. This water is then run on the corn and recirculated at a temperature of 125°F for 36 to 48 hours to remove corn solubles from the corn berry. At the completion of this steeping process, the water is drawn off for concentration. The Baumé varies from 4° to 6° according to the specific steeping system in use. Concentration is made to 25° Bé or better and the finished product, now known as *steep water*, is ready for blending into feed.

The corn from the steeps passes through a Foos mill, which shreds the berry, leaving the germ relatively intact. The germ is then floated off, washed, dried, and pressed for its oil. The cake when ground is sold as *corn oil meal*.

The hull, fiber, corn gluten, and starch remaining contain a large quantity of grits. In order to separate the starch from the grits, the material passes to a Buhr mill. From the Buhr mill the stream passes through a series of coarse copper reels which separate and wash the *hull*, or *bran*. This material is expressed and dried. It is then ready as a component for compounding into gluten feed.

The effluent now contains starch, corn gluten, and fine fiber. The stream passes a series of reels, made of bolting silk, wherein fine fiber is separated, washed, and collected wet for blending. ^{note}

known as *fine fiber* or *No. 17 tailings*, the latter name being derived from the silk used.

The effluent now contains starch and corn gluten. The stream passes to the tables where starch is removed by sedimentation and the gluten passed to settling tanks. After a period of settling, the supernatant liquid is decanted and the heavy gluten slurry passed to Merco centrifugals which remove a further quantity of starch which has escaped the tabling operation. The *corn gluten* is then resettled and pressed, usually as a mixture with the fine fiber, which reduces the protein content to the minimum requirements for gluten meal.

The amounts of the end products vary with the company. An approximate breakdown of a bushel of corn is as follows:

	Lbs
Water.....	8.4
Starch.....	33.0
Oil.....	1.5
Corn oil meal..	1.8
Hull or bran ..	2.0
Fine fiber	2.3
Gluten....	3.5
Steep water solids	3.5
	56.0

The composition of these products expressed on a dry-substance basis is approximately as follows:

	Hull	Fine fiber	Gluten	Corn oil meal	Steep water solids
	%	%	%	%	%
Crude protein.....	7.1	14.7	67.5	25.7	45
Crude fat.	2.3	1.4	5.7	12.7	—
Crude fiber.	19.4	8.5	2.2	9.8	—
Ash.....	0.8	1.0	2.0	2.9	18
Starch	6.2	49.1	15.0	14.2	—
Pentosans.....	64.2	25.3	7.6	34.7	—
Gums and sugars ¹ ..	—	—	—	—	37

¹ By difference.

- This paper is divided into three sections covering the moisture content of corn hulls, steep water, and corn gluten—the three products which are used in the compounding of the commercial feeds.

Determination of Moisture in Hull and Fine Fiber

Basically, hulls and fine fiber are the same except for degree of subdivision. As a result, a moisture study was conducted solely with ^{and} hulls. The methods for moisture analysis were similar to those described in previous papers. The complete data are given in Table I.

TABLE I
THE MOISTURE OF CORN HULLS

Moisture method	Temperature	Length of drying showing period of essential constancy	Moisture range during period of essential constancy	Final moisture value
	°C	hr.	%	%
De Bruyn	38	70-114	6.70-6.77	6.77
	60	70-114	6.74-6.78	6.78
	93	70-114	6.79-6.77	6.79
Distillation	Benzene, bp 80°C	5- 7	6.80-6.82	6.82
	Toluene, bp 110°C	5- 7	6.78-6.80	6.80
Vacuum oven	80	16- 45	6.80-6.83	6.83
	110	16- 45	6.77-6.79	6.79

The moisture data for hulls show that the material is stable to drying and that the water is removed rapidly and completely. There can be no doubt that the true moisture is obtained since the various diverse procedures yield results in agreement. These results demonstrate that in the compounded feedstuffs, the hull or fine tailings fraction can be considered as a stable ingredient in which the true moisture can be readily determined.

Determination of Moisture in Steep Water

Practical experience of laboratories in the industry has indicated that steep water is very unstable. The instability of gluten feed during storage, which will be dealt with in a subsequent paper, has been attributed to the steep-water fraction. The nitrogen in steep water calculated as protein (factor 6.25) approximates 50% of the total solids. Inasmuch as the nitrogen fraction is largely composed of hydrolytic products including amino acids, the percentage of nitrogen compounds is probably greater than indicated by the use of the factor 6.25. Steep water also contains a high percentage of ash (15%-20%), some of which is combined with phytic acid. The remainder is largely reducing sugars.

A cold-water extract (0°C) of corn removes approximately 3.9% (DS basis) of soluble material from the corn berry. This is equivalent to approximately 1.9 pounds per bushel. Actual milling practice results in two to three times this amount. This increase is largely in the crude protein fraction as a result of acid hydrolysis in the steeps, together with fermentation and enzymic action in the process. A large percentage of the crude protein fraction is amino acids, which in combination with the sugars represent a product which may be expected to be unstable to drying.

All determinations of steep-water solids by the industry have been considered as approximate, since it is well recognized that the value obtained was dependent on the method of drying. The methods used in the determination of true solids and true moisture of this product will therefore be given in detail, as an example of the method of attack used in the determination of moisture on extremely thermolabile products such as steep water.

Preparation of sample: A composite sample of all heavy steep water produced over a two-day period was made. The pH of the material was 4.32 as determined by glass electrode. The Baumé was approximately 25°. Since steep water of this density carries suspended material, every effort was made to insure uniformity in sampling for the various tests. All weighings for the various determinations were made on the same day.

De Bruyn method: The De Bruyn method was originally applied by Cleland and Fetzer (1942) to the determination of moisture, at low temperatures and high vacuum, in corn sirup, which was then thought to be a heat-sensitive material. Filter Aid was introduced with the viscous material in order to disperse the sample over a very thin layer, from which moisture could be removed readily and completely. Steep water is an analogous material in that, on drying, the mass becomes viscous and gluey, from which moisture is removed with difficulty. Although the De Bruyn determination was designed for low temperatures, the tests on steep water covered a wide range of temperature to permit observation of changes that occurred in the sample during drying. Drying was conducted at 40°, 50°, 60°, 80°, and 95°C.

About 30 g of Filter Aid was run into a De Bruyn flask, test tube inserted, and the same dried in a vacuum oven at 100°C for five hours. The flask was then capped, cooled in a desiccator, and weighed in a tared flask. To the weighed flask was added approximately 20 g of the steep water and the flask reweighed for sample weight. The steep water was then dispersed in the Filter Aid by means of a glass rod which fitted snugly into the test tube. The flask was then attached to the connecting arm of the De Bruyn, an empty flask being placed on the other end and then attached through a large calcium chloride trap to a vacuum pump. After the system had remained overnight at room temperature, the mass of steep water and Filter Aid was slightly damp. At this time the flask was removed and the damp mass worked completely into a uniform, powdered mass. It was then reconnected to the connecting arm and approximately 30 g of P_2O_5 was introduced into the other flask. The De Bruyn was then evacuated by a vacuum pump (Megavac) and the flask containing

the sample was placed in an insulated box arranged with electric lights to give the temperature desired, while the other flask containing P_2O_5 was placed in a bath of running cold water. Cello-Grease (Fisher Scientific Co.) was used for sealing the joints. At the end of 24 hours the vacuum was released by dry air passing through a train containing Drierite and P_2O_5 , and the flask containing the sample weighed. The flask was reattached to the connecting arm and a new flask with fresh P_2O_5 attached to the other end. The De Bruyn was again evacuated to 0.1 mm or less and allowed to run for a two- or three-day period before it was again reweighed. Both flasks were rotated daily in order to expose fresh surface. The P_2O_5 flask was renewed as needed. *The surface must remain free-flowing.* Drying was continued until constancy in weight was obtained.

Vacuum oven methods: The following pieces of apparatus were used:

Flasks—250-ml Erlenmeyer standard taper 40/50. These flasks are used interchangeably for oven work and as distillation flasks for distillation work described in previous papers.

Stoppers—Pyrex standard taper 40/12.

Test tubes—Pyrex 100 × 13 mm. Used as pestles for reducing Filter Aid sample mass to a powder. These are weighed with the flask and Filter Aid, and are used as a pestle by the insertion of a glass rod.

Twenty grams of steep water was added to a previously vacuum dried flask containing Filter Aid. The sample was dispersed in the Filter Aid by means of the pestle. It was then given a period of drying in the vacuum oven, after which the sample was reworked to a fine powder. The temperatures employed were 60°, 70°, 80°, and 90°C.

Distillation methods: Apparatus for the distillation methods was similar to that described in previous papers for corn. Approximately 8 g of steep water was run into a weighed flask containing Filter Aid and pestle, which had been previously vacuum dried at 100°C. The flask was reweighed in a tared flask. The sample was quickly incorporated with Filter Aid, 75 ml of solvent was added, and the sample was reworked. The flask was attached to the trap and condenser, the trap filled with solvent, and distillation started slowly.

De Bruyn results: The apparent moisture values obtained by drying steep water in De Bruyns at varying temperatures is shown in Figure 1. After 350 hours of drying, the comparative moisture values obtained were as shown in Table II.

The drying of steep water for 350 hours at 80°C resulted in a loss of dry substance equivalent to 8.9%. Drying at 95°C resulted in a loss of 20.5%. At the lower temperatures, decomposition occurred, much more slowly. The De Bruyn at 50°C continued to show a gradual weight change over the total drying period of 900 hours in
it be

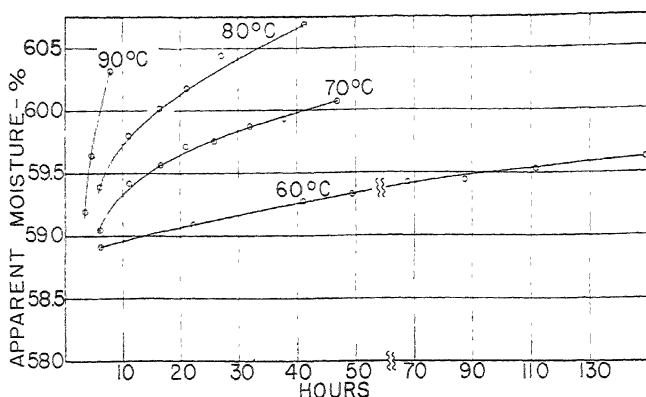


Fig. 1. The drying of steep water in the De Bruyns.

TABLE II
THE DRYING OF STEEP WATER IN DE BRUYNs

Temperature of drying	Apparent moisture	DS decomposition ¹
°C	%	%
40	59.3	0.00
50	59.4	0.00
60	60.4	2.2
80	63.1	8.9
95	67.8	20.5

¹ The value 59.5% was taken as a measure of the true moisture content of this sample of steep water in the light of subsequent work to follow.

At 40°C, constancy in weight was reached. Specific observations on the flasks at various temperatures are as follows:

Drying at 80°C and 95°C: Large quantities of volatiles condensed on the walls of the De Bruyns, particularly at the higher temperature. The Filter Aid steep water mixture became brown to black. At the 95°C temperature, the volatile oil partially crystallized, some fine white needles being formed.

Drying at 60°C: No sign of volatiles noted. The Filter Aid became yellowish within the 90-hour drying period, after which the color slowly increased to light brown (250 hours). The phosphorus pentoxide remained colorless.

Drying at 38°C and 50°C: At the end of the 200-hour drying period, a faint yellow tinge was present at the 50°C temperature, which very slowly increased in hue, and after 900 hours of drying the Filter Aid became yellow.

Vacuum-oven results: The vacuum-oven data for steep water are shown in Figure 2. The samples were removed from the oven, cooled, and weighed at the periods indicated in the graph. As can be observed, the data obtained at the higher temperatures—70°, 80°, and 90°C—compare closely with those obtained in the De Bruyns evaporated temperatures. The continued marked weight loss with

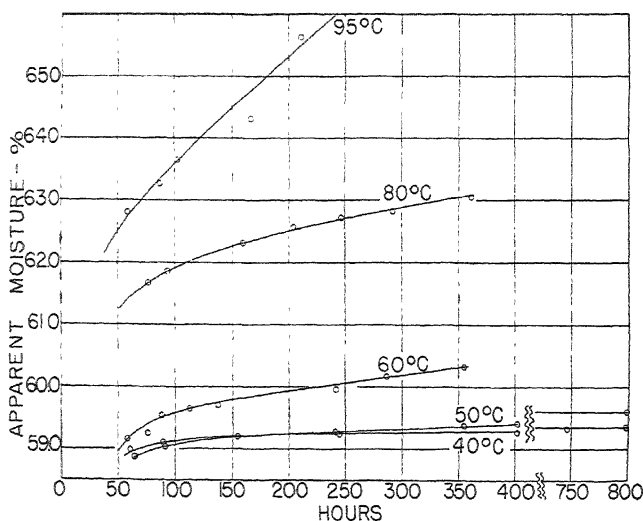


Fig. 2. Vacuum-oven drying of steep water

drying indicates decomposition. At 60°C, the loss in weight for the first 60 hours was quite rapid, after which the samples continued to lose weight at a very slow rate (0.08% per 44-hour interval). The uniformity in loss in weight suggested that a slow decomposition was in progress.

Distillation results: The distillation data for steep water are given in Table III. With benzene, the water obtained reached a constant value within 8 hours, whereas with the benzene-toluene mixture and toluene, constancy was not reached after 18 hours of distillation. An examination of the flasks after distillation showed only slight color

TABLE III
DETERMINATION OF MOISTURE IN STEEP WATER BY DISTILLATION METHODS

Length of distillation, hr.	Solvents		
	Benzene, bp 80°C	Benzene-Toluene mixture, bp 92°C	Toluene, bp 111°C
1	45.80	55.19	53.67
2.25	57.10	60.96	59.63
3.00	58.42	61.09	60.97
6.00	58.55	61.46	61.27
8.00	59.48	61.59	61.42
16.00	59.48	61.84	62.18

rs.

formation with benzene, while the mixture of Filter Aid and steep-water solids for the other two solvents had become very dark brown, indicative of decomposition. As will be shown in the next section, the results indicate that the use of benzene is satisfactory for the estimation of the true moisture in a product as unstable as steep water. Toluene distillation, therefore, resulted in an increase of 2.70% of apparent moisture, which on the basis of dry solids is equivalent to the formation of 6.7% of water of decomposition.

Discussion of the moisture methods for steep water: Of all the conditions used, only four methods yield values in which the question of decomposition is limited to a minimum. These values are summarized in Table IV.

TABLE IV
MOISTURE METHODS FOR STEEP WATER

Method	Length of drying hrs	Moisture content %
De Bruyn 40°C	950 (constancy)	59.38
50°C	357	59.38
	447	59.47
	677	59.61
	900	59.65
		Av 59.53
Vacuum oven 60°C	78	59.42
	98	59.43
	122	59.52
	170	59.61
		Av 59.50
Benzene distillation	16 (constancy)	59.48

In the case of the De Bruyn at 50°C and the vacuum-oven method at 60°C, no constancy in weight was obtainable within a reasonable time interval, and the material was slowly coloring (yellow), which is an indication of decomposition. The moisture values over a lengthy drying period were, therefore, averaged as an estimate of the moisture content. This procedure cannot result in very large error, since the total weight loss during the final 100-hour drying period at 60°C in vacuum was only 0.19%, and in the De Bruyns the change occurring during the drying period from 357 hours to 900 hours was 0.27%.

The two methods yielded constant results, and it is of interest to note that the results are in agreement by 0.1%. The De Bruyn method of drying steep water at 40°C yielded a value agreeing within 0.1% of that obtained by the direct determination of water by benzene distillation. The distillation method also agrees with the De Bruyns at 50°C (0.05%) if the moisture values obtained for the 357- and at 900-hour periods are averaged, and also with the vacuum-oven results

at 60°C. The benzene distillation method has been adopted as the official reference method for the determination of water in steep water by the Technical Advisory Committee of the Corn Industries Research Foundation.³

Determination of Moisture in Corn Gluten

A composite sample of corn gluten leaving the Merco was filtered and washed exhaustively with water to free the material from the unstable gluten water and solubles. The material was then air dried and ground to pass a 20-mesh sieve. Moisture studies were confined to distillation methods, oven methods, and a single reversibility study. These limited tests were deemed sufficient, inasmuch as a more complete study was under way with gluten meal (corn gluten and fine fiber). The paper on gluten meal follows next in this series. The moisture data are given in Table V.

TABLE V
THE MOISTURE OF CORN GLUTEN

Moisture method	Temperature	Final period of drying	Moisture range during final drying period	Final moisture value
Distillation	°C	hrs	%	%
	80 Benzene	42-65	8.58	8.58
	110 Toluene	42-65	8.66	8.66
Vacuum oven	80	16-45	8.58-8.67	8.67
	110	16-45	10.06-10.50	10.50

The final constant distillation values for benzene and toluene were 8.58% and 8.66%, a difference of 0.08%. As pointed out in the previous papers, the now accepted A. O. A. C. (1940) official method for the determination of moisture in feeds by toluene distillation states that the distillation is usually over within one hour. At that period, the obtained moisture value for the corn gluten was 8.20%, which is 0.46% lower than that found with continued distillation. The present procedure of limiting the distillation to one hour undoubtedly results in low moisture values.

The vacuum-oven results at 80° and 110°C show considerable disparity. In neither case was constancy in weight obtained, and the apparent moisture values differed by 1.83%. In a second study, the corn gluten was dried *in vacuo* at 100°C, and a continued weight

³ The above studies were conducted with steep water concentrated directly after removal from the steeping tanks. If the steep water is allowed to undergo partial fermentation prior to concentration, the development of volatile acids may require their partial neutralization before distillation with benzene.

loss with drying was obtained with values intermediate between those previously found. These results again show, as was found for corn, that it is just a question of chance whether oven results at two different temperatures will or will not agree.

The corn gluten dried *in vacuo* at 80°C yielded an apparent moisture value which was 1.83% lower than that obtained by drying the material *in vacuo* at 110°C. Whether or not this marked difference at the elevated temperatures is due to moisture loss or to the loss of volatiles or products of decomposition cannot be determined by the oven data. The toluene distillation method yielded a moisture value of 8.66%, which agrees with the 80°C vacuum value after a drying period of 45 hours, and this agreement suggests that the higher vacuum values at 110°C are not caused by water loss but rather by a dry substance loss. Previous work with corn (see previous papers) has shown that vacuum drying of corn at 110°C for 30 hours results in a volatile loss of 0.60%. Inasmuch as corn gluten contains a higher percentage of ether extract than corn (approximately 50% greater) vacuum drying of this product would be expected to yield an even greater dry substance loss. That this is the case is shown by the following reversibility study.

A full discussion of the reversibility method and its use in the determination of the true moisture content of cereals will be dealt with in the next paper of this series, covering the moisture of gluten meal. A brief outline of the principles was given in the first paper of this series, but a full discussion was reserved for the gluten meal moisture study, since the method was originally applied to that product.

The corn gluten samples dried *in vacuo* at 80° and 110°C, along with control untreated samples, were allowed to reach equilibrium in the Weber oven at 80°C. A stream of dry nitrogen was passed through the oven, in order to eliminate humidity fluctuations. Equilibrium was reached within 48 hours, and the results obtained are given in Table VI.

The reversibility data indicate that the vacuum drying of corn gluten at 80°C results in a slow decomposition or volatile loss. This observation was previously noted for corn. At 110°C *in vacuo*, the decomposition is very marked. By correcting for the decomposition, it is found that the calculated true moisture for the 80° and 110°C vacuum results is 8.48% and 8.59%, respectively. In other words, when the apparent moisture content of corn gluten was 8.67% (80°C *in vacuo*) this value included 8.48% of water and 0.19% of volatiles. At 110°C, the apparent moisture value of 10.50% included 8.59% of water and 1.91% of volatiles. This latter moisture figure is in agree-

TABLE VI
REVERSIBILITY AND MOISTURE LOSS OF CORN GLUTEN

Moisture method	Temperature	Length of drying	A Apparent moisture	Reversibility study			
				B Reversible moisture value, 80°C, N ₂ 48 hrs	C Adsorption A-B	D Decomposition ¹ B-8.25	E Calculated true moisture A-D
Control air oven, N ₂	°C	hrs	%	%	%	%	%
	80	40	8.27	8.25	—	—	—
Vacuum oven	80	45	8.67	8.44	0.23	0.19	8.48
Vacuum oven	110	45	10.50	10.16	0.34	1.91	8.59

¹ Includes volatile loss

ment with the distillation results. The lower moisture value indicates that the 45-hour drying period at 80°C was insufficient for the total removal of the water.

Summary

The moisture content of the three mill products which are used in the compounding of the commercial feedstuffs in the corn industry was determined.

The hulls or fine tailings are stable, and the true moisture content can be determined by a variety of methods which includes distillation with benzene or toluene, drying in a De Bruyn over P₂O₅ at temperatures ranging from 40° to 90°C, and by vacuum drying at 80° or 110°C.

Steep water is an unusually thermolabile product, which undergoes decomposition at low temperatures (60°C). The moisture cannot be removed directly from this product by oven or distillation methods, since on drying it becomes extremely viscous and severe case-hardening occurs. It is necessary first to disperse the material on Filter Aid. The material can then be dried to constancy by two moisture methods, one direct and the other indirect, which are in agreement. The values obtained by drying the material to constancy in De Bruyns at 40°C agree with the direct determination of moisture by benzene distillation. Toluene distillation of steep water yields higher moisture values, equivalent to 6.7% of water of decomposition. The benzene distillation method has been accepted as the official reference method for the determination of the true solids in steep water by the Technical Advisory Committee of the Corn Industries Research Foundation.

The true moisture in corn gluten cannot be satisfactorily determined by oven methods. Vacuum drying of this product results

marked loss of volatiles (1.91% at 110°C). The toluene distillation method is satisfactory for the determination of moisture in corn gluten.

Literature Cited

- Association of Official Agricultural Chemists
 1940 Official and tentative methods of analysis of the Association of Official Agricultural Chemists (5th ed.).
 Cleland, J. E., and Fetzer, W. R.
 1941-42 Determination of moistures in sugar products. II. Corn products. Ind. Eng. Chem. (Anal. Ed.) 13: 855-860 (1941); 14: 27-30; 124-129 (1942).

THE DETERMINATION OF MOISTURE IN THE WET MILLING INDUSTRY. IV. CORN GLUTEN MEAL

L. SAIR¹ and W. R. FETZER²

This paper is a continuation of a series dealing with the moisture in corn and its feed by-products. The first two papers dealt with corn, while the third dealt with the moisture study of the blending components—namely, the hulls, corn gluten, and steep water, which make up the commercial by-product feeds. It was thought that a study of these constituents would lead to a better understanding of the moisture determination in the compounded feedstuffs. Three commercial feed products produced by the corn industry are sold under the trade names of corn gluten meal, corn gluten feed, and corn oil meal. This paper deals with a moisture study of gluten meal.

In the wet milling process, the corn gluten is separated from the starch by tabling, the fraction floating off being termed corn gluten. The slurry is then passed to a centrifugal which reduces the starch content to approximately 20%. In the wet stage, this material is mixed with tailings (fine fiber) and is run through either a filter press or string filter, after which it is kiln dried. The dried product is gluten meal. A typical analysis follows:

	%
Moisture	7.6
Crude protein	43.8
Crude fat	0.70
Ash	1.2
Fiber	3.8
NFE (by difference)	43.6

Studies were made on composite samples of corn gluten meal produced by two members of the industry. The methods of moisture approach were essentially similar to those previously described for corn, which included distillation methods, oven methods, the De Bruyn

¹ Research Fellow, Corn Industries Research Foundation.

² Chief Chemist, Union Starch and Refining Co., Granite City, Illinois.

method of drying in high vacuum over P_2O_5 , and the reversibility method. Both samples were ground to pass a 20-mesh sieve.

In all cases, the difference between duplicates includes the sampling error, since the duplicate samples for each determination were taken from different bottles of gluten meal. The individual values for all determinations with the exception of the oven results are included in this paper. The difference between the duplicate values for the oven determinations rarely exceeded 0.03%, the SE from the means for the duplicates being 0.02%.

Moisture Content of Gluten Meal No. 1

The moisture results obtained by the use of distillation methods, De Bruyn methods, and by oven methods for the first sample of gluten meal (No. 1) tested are given in Table I. It should be made clear

TABLE I
THE MOISTURE OF GLUTEN MEAL NO. 1

Moisture method	Temperature	Length of drying, showing period of essential constancy	Apparent moisture		
			Moisture range during period of essential constancy	Final moisture value	
				Duplicates	Average
	°C	hrs	%	%	%
De Bruyn	40	240-340	7.80-7.84	7.84	7.84
	60	240-340	7.81-7.83	7.85-7.81	7.83
	90	80-200	7.89-7.92	7.90-7.94	7.92
Oven drying	Vacuo 80	60-160	7.86-7.88	7.89-7.88	7.88
	90	16-60	7.88-7.94	7.94-7.95	7.94
	100	11, 24-80	7.86-7.90	7.88-7.92	7.90
		(2, 16-60	7.89-7.90	7.91-7.89	7.90
	120	40-90	8.12-8.16	8.16-8.17	8.16
	Air 100	5-160	7.42-7.38	7.36-7.39	7.38
	130	24-60	7.94-8.01	8.03-7.99	8.01
Distillation	Benzene	30-48	7.76	7.69-7.76-7.75 7.82	7.76
	Toluene	30-48	7.87	7.78-7.84-7.92 7.94	7.87

that for each moisture procedure, the test was continued until an essential equilibrium value was obtained; that is, for each condition of temperature, pressure, desiccant or solvent, drying was continued until a constant value characteristic of the apparent moisture resulted.

The data given in Table I show that the gluten meal sample was markedly stable to heat, which is surprising in view of the results previously obtained for corn gluten (see paper No. II). Vacuum drying of corn gluten at 80° and 110°C resulted in an apparent moisture

difference of 1.8%. This is attributed, in view of the similar moisture values obtained by benzene and toluene distillation, and also reversibility studies, to volatile loss. Corn gluten contains 5.5% to 7.0% of fat. With gluten meal, this marked difference in oven data does not occur, thus indicating a loss or fixation of the volatiles during kiln drying. Analytical data indicate that some change does occur during drying, since the ether extract values for corn gluten and gluten meal approximated respectively 6.0% and 1.0%. The dilution of corn gluten with fine tailings in the preparation of gluten meal amounts to approximately 40%, which in terms of ether extract should reduce the fat value in the gluten meal to 3.6%. This discrepancy in fat content of the gluten meal is attributable largely to a fixation within the protein meshwork and because of case-hardening during kiln drying. If gluten meal is first subjected to a mild acid treatment prior to ether extraction, a higher ether extract value is obtained. The effect of drying, therefore, increases the stability of gluten meal with respect to volatile loss during oven drying.

The moisture range obtained by the various moisture methods varied from 7.38% (air oven, 100°C) to 8.16%. The low air-oven values are attributable to the property in gluten meal of retaining water at this temperature, as will be shown in a following section. The significant moisture range can be narrowed to include values between 7.76% and 8.16%, a difference of 0.40%.

The drying of gluten meal in De Bruyns at high vacuum (0.01 mm) over P_2O_5 was conducted at 40°, 60°, and 90°C. It will be noted that a 50°C differential in temperature resulted in only an 0.08% increase in moisture. The obtained moisture range varied from 7.84% to 7.92%.

Vacuum drying was conducted at 80°, 90°, 100°, and 120°C. The final moisture values obtained for the 80°, 90°, and 100°C results were within very narrow limits, a difference of only 0.06% being obtained. The values ranged from 7.88% to 7.94%, which are in essential agreement with those obtained with the De Bruyns. At the 120°C temperature, the obtained moisture value shows an abrupt increase equivalent to 0.26%. Inasmuch as the 80° and 100°C values were essentially similar, this increase at 120°C suggested decomposition. That this was the case will be shown later in the section of this paper dealing with reversibility.

Distillation with benzene and toluene yielded results which differed by 0.11%. This may be explained in the method of drying of this product. The material, by impact, often dries to a hard horny mass. Even with the material ground to 20-mesh, it might be expected that the low boiling solvent (benzene 80°C) might find it difficult to remove

all the water. The values obtained by toluene distillation (7.87%), however, agree with the vacuum oven results at 80°, 90°, and 100°C and with the De Bruyn values at 90°C.

The results, as given in Table I, indicate that the moisture values fall roughly into three groupings—a lower moisture range of 7.76% to 7.85%, an intermediate group varying from 7.87% to 7.94%, and higher values extending from 8.01% to 8.16%. The lower results were obtained by subjecting the gluten meal to mild conditions, the feed being dried in De Bruyns at 40° and 60°C or distilled with benzene. The higher moisture values were obtained by subjecting the gluten meal to a relatively drastic treatment. In one case, the feed was dried for 60 hours in an air oven at 130°C. A second procedure involved vacuum drying for 90 hours at 120°C. The intermediate moisture values include the De Bruyn drying at 90°C, vacuum drying at 80°, 90°, or 100°C, and toluene distillation.

Studies which will be given in the next section throw considerable light on the problem of determining which of the above moisture groups is representative of the true moisture content of gluten meal.

Moisture loss and reversibility: The principles underlying the application of the reversibility method for the determination of the true moisture content of corn were briefly outlined in the first paper of this series. Since this method was originally used during the investigations conducted with gluten meal, a more lengthy discussion of the principles and uses of the reversibility method will be given here.

Van Bemmelen (1910) in his studies on the moisture relations of silicic acid gels showed that the desorption (moisture loss) and adsorption (moisture gain) curves did not coincide over the whole moisture range. The desorption and adsorption curves coincide for the first increment of water adsorbed, after which the previously dried gel shows a diminishing ability to adsorb moisture with increasing moisture content, as compared to the original gel. This behavior has been termed hysteresis. Urquhart and Williams (1925) have obtained essentially similar results with cotton. They found that the adsorption and desorption curves coincide, when dried cotton (vacuum oven, 110°C) is allowed to adsorb only 1% of water. Beyond this, the adsorption curve shows an increasing variance with the desorption curve with increase in moisture content. The heat-treated cotton loses its ability to hold as much water. Pidgeon and Maass (1930) have reported essentially similar results for cellulose. The two curves coincide for the first moisture uptake, after which hysteresis occurs.

The findings of these investigators offer the basis upon which the reversibility method has been developed for the determination of the true moisture content in cereals. They found that the drying of

silicic acid gels, cotton, and cellulose is completely reversible if the dried materials are allowed to readsorb only 1% of moisture. If during drying the products have undergone some decomposition, or if a volatile has been removed, then this complete reversibility is unobtainable. It was thought that if this behavior could be found applicable to cereals, it would be of value in the determination of moisture because decomposition or the loss of volatiles could then be detected.

Davidson and Shorter (1930) made use of the desorption-adsorption relation in the determination of moisture in cotton. They dried cotton at 90°, 100°, 110°, and 120°C and then redried all the products at 90°C after moistening. Equilibrium values in excess of those obtained by cotton dried directly at 90°C were considered to be attributable to decomposition. In a second study, cotton was dried at temperatures of 100°, 135°, 160°, 182°, 200°, and 212°C. These products were then redried at room temperature over P_2O_5 , and the obtained dry-substance value was compared to a control dried directly over P_2O_5 . Davidson and Shorter concluded that the true moisture of cotton is obtainable, and pointed out that their results were not in agreement with those obtained by Nelson and Hulett (1920).

The desorption-adsorption method for the determination of moisture was applied to corn (No. I of this series) and the calculated true moisture was found to be in agreement with that obtained by other moisture methods, thus indicating the utility of this procedure.

The data obtained for gluten meal indicate that for each condition of temperature, pressure, and desiccant, the material attained an equilibrium value. For example, in an air oven at 100°C, gluten meal attained constancy during the period from 5 to 140 hours, the moisture value ranging from 7.33% to 7.43%. This apparent moisture value fluctuated from day to day within this narrow range, showing no particular trend. Inasmuch as the 5- and 140-hour period values are in close agreement, these results preclude the possibility of decomposition with a resultant loss in weight. Therefore, it would be expected that this particular sample of unaltered gluten meal should, irrespective of its previous drying treatment, attain a value of 7.40% when allowed to reach equilibrium at 100°C in air. This value, in a sense, can be considered as a *physical* constant. Similarly, it was found that the gluten meal when dried in a vacuum oven at 100°C attained an equilibrium at 7.90%.

The following experiments were carried out which showed that the values obtained in the air and vacuum ovens at 100°C were completely reversible:

1. When the vacuum-dried gluten meal was replaced in the air oven, it adsorbed 0.5% of moisture and reached the apparent moisture value of 7.40%, characteristic of unaltered gluten meal.

2. When air-dried gluten meal was placed in the vacuum oven, it lost sufficient moisture to yield a value of 7.75%. This value is still 0.15% too low as compared to direct vacuum drying of gluten meal. That this difference was due to "case-hardening" in the air oven was proved by moistening the air-dried samples prior to transfer. By first softening the feed, the vacuum oven values were increased to 7.88%, a value similar to that obtained by drying the feed direct.

These results show that gluten meal can adsorb at least 0.5% of moisture reversibly and that in this respect, gluten meal behaved in a manner similar to silicic acid, cotton, and cellulose. Moreover, it follows that the vacuum-oven value at 100°C included no losses due to decomposition or the loss of volatiles.

The gluten-meal samples dried for 60 hours at 130°C in an air oven (8.01%) were treated similarly. In this case, the feed reached an equilibrium value of 7.88%, which is a value far removed from that obtained with unaltered gluten meal (7.40%). These results indicate that the heating of gluten meal at 130°C for a long period caused some physical or chemical changes, thus reducing its water-binding capacity. It was thought that possibly the failure for reversibility might be caused by an unusual case-hardening of the material at the high temperature. Therefore the samples were moistened and replaced in the air oven at 100°C. An apparent moisture value of 7.86% was obtained, thus eliminating the possibility that this factor was responsible for the differences found.

Various other experiments of a similar nature have been carried out, and the data are given in Table II. These data indicate that the moisture-loss curve of this sample of gluten meal is completely reversible if the apparent moisture obtained does not exceed 7.88%. Beyond this, complete reversibility is not obtainable, the reversible value being greater than 7.40% to almost exactly the same amount as the apparent moisture value exceeded 7.88%. In all cases, the samples adsorbed essentially the same amount of water, 0.46%–0.50%. It appears, therefore, that the values greater than 7.88% are due to losses attributable either to the loss of volatiles or to the loss of decomposition products.

By subtracting the percentage of decomposition obtained by the various vacuum-oven treatments from the apparent moisture, it can be noted that the calculated true moisture values fall within a very narrow moisture range, 7.86%–7.90%.

TABLE II
 REVERSIBILITY AND MOISTURE LOSS OF GLUTEN MEAL

Moisture method	Temperature	Interval of drying	Reversibility study				
			A	B	C	D	E
			Apparent moisture	Reversible moisture value at equilibrium in air oven at 100°C (72 hrs)	Adsorption of water A-B	Decomposition ¹ B-7.40%	Calculated true moisture A-D
Control air oven	°C	hrs	%	%	%	%	%
	100	24	7.38	7.40	—	—	—
Vacuum oven	100	21	7.88	7.39 7.41 Av 7.40	0.48	0.00	7.88
Air oven	130	4	7.88	7.43	0.46	0.02	7.86
Vacuum oven	100	16		7.42 Av 7.42			
Vacuum oven	90	60	7.94	7.42 7.46 Av 7.44	0.50	0.04	7.90
Vacuum oven	100	64		7.52	0.50	0.10	7.90
	120	4	8.00	7.48 Av 7.50			
Vacuum oven	100	64		7.69	0.48	0.28	7.88
	120	90	8.16	7.68 Av 7.68			
Vacuum oven	120	90	8.18	7.68 7.69 Av 7.68	0.50	0.28	7.90
							Av 7.89

¹ Decomposition includes the loss of volatiles

Discussion of the results obtained for gluten meal No. 1: The data given in the foregoing section indicate that the true moisture content of the sample of gluten meal under examination lies within the limits of 7.86% to 7.90%. Gluten meal dried to constancy in a vacuum oven gave the following results:

Temperature of oven °C	Apparent moisture %
80	7.88
90	7.94
100	7.90
120	8.17

The moisture results obtained at 80°, 90°, and 100°C are in essential agreement. At the higher temperature (120°C) a distinct break occurs, resulting in an apparent increase of 0.27% in moisture. It is generally recognized that water is held by colloidal materials with different degrees of tenacity and hence, for each increase in temperature, more moisture is liberated if additional water is present. This was not the case for this sample of gluten meal, and therefore the break must indicate some loss other than water held by purely me-

chanical or physical forces. This might be explained as caused by water loss, but if so this water is held by forces of an order different from the water removed at the lower temperatures.

The De Bruyn method of drying *in vacuo* over P_2O_5 yielded moisture values ranging from 7.83% to 7.92%. The reasons for the slightly lower results obtained at 40° and 60°C (7.83%) are undoubtedly the same as was previously reported for corn ground to only 10-mesh (No. 1 of this series). The hard grits reduce the availability of the water when dried at low temperatures. As a consequence, a false equilibrium results. This will be shown in more detail in the second study of a gluten meal.

Toluene distillation yielded a moisture value of 7.87%. This method determines water directly and the value obtained is in agreement with the vacuum-oven results (7.88%–7.94%), the De Bruyn method at 90°C (7.92%), and with the reversibility studies (7.89%). The results of this investigation show that the toluene distillation method, when carried to completion, removes the total water from gluten meal.

The Moisture Content of Gluten Meal No. 2

Upon completion of the study on gluten meal No. 1, the work was repeated on a second sample composited by a different company. The object was to determine the variability that might exist from the moisture standpoint in gluten meals produced by different commercial processes.

The moisture content of the gluten meal was determined by vacuum oven drying at 90°, 100°, 110°, and 120°C, by air drying at 100°C, by the toluene distillation method, and by reversibility studies. A summary of the oven and distillation data is given in the following table, and shown in Figure 1.

TABLE III
THE MOISTURE OF GLUTEN MEAL No. 2

Moisture method	Temperature	Interval of drying	Moisture range	Maximum apparent moisture value
	°C	hrs	%	%
Air oven	100	24–96	10.98–11.04	11.04
Vacuum oven	90	16–40	11.20–11.24	11.24
	100	16–72	11.40–11.49	11.49
	110	8–40	11.47–11.64	11.64
	120	2–16	11.41–11.68	11.68
Toluene distillation	bp 110	4–28	11.39	11.40 11.37 11.39 Av 11.39

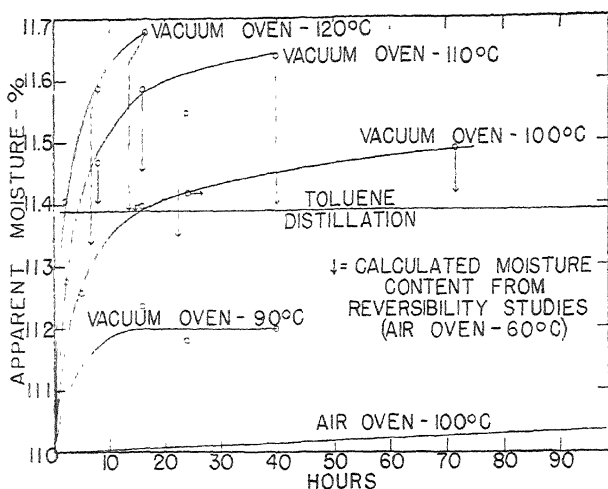


Fig. 1. The determination of moisture in gluten meal, No. 2

The results obtained with the second sample show a considerable variation from those previously obtained. The first sample was much more stable, the meal exhibiting a constancy in weight when heated *in vacuo* at 100°C for a considerable period. A difference of only 0.30% was obtained between vacuum drying at 80° and 120°C. With the second sample, the difference between 90° and 120°C amounted to 0.44%, even though the material was heated for only 16 hours at the higher temperature in contrast to 90 hours for the first sample. That this variability occurs can be readily understood when it is realized that the gluten water from which the corn gluten settles varies markedly in stability, dependent on the conditions under which it is made. Corn gluten, as it leaves the string filter, contains 60% of gluten water, which, in turn, contains approximately 2% of solubles. The dried gluten, therefore, contains 1.2% of gluten-water solubles. A second variability resides in the method of drying the gluten meal. Previous work (paper No. II) showed that corn gluten contains material which readily volatilizes *in vacuo* at higher temperatures. These volatiles were apparently firmly fixed in gluten meal No. 1 but only partially in the second sample.

The data in Table III indicate a different apparent moisture value for each oven condition. At 90°C *in vacuo*, constancy was reached at a moisture value of 11.24%, the value shifting only 0.04% in 24 hours. At 100°C the moisture value was increased to 11.49%.

the value increasing 0.09% in the last 56 hours of drying. The lower value, as will be shown later, is attributable to severe case-hardening, which may readily occur in kiln drying, with a resultant false equilibrium. At the higher drying temperatures (110°–120°C) the values showed a constant increase. Toluene distillation yielded a constant value of 11.39%.

From the above results it is impossible to make any deductions regarding the true moisture content of the feed sample. It might be surmised that the true value lies in the region of 11.40%–11.49%, since with continued heating for 56 hours *in vacuo* at 100°C, the value shifted only 0.09%. However, this assumption would at best be a compromise, since it might be argued that since constancy was reached *in vacuo* at 90°C, the moisture value of 11.20%–11.24% should be chosen.

The reversibility method has been of particular value in clarifying the oven data. The moisture dishes containing the feeds dried *in vacuo* at 100°, 110°, and 120°C were compared to unheated gluten meal samples at a temperature which precluded decomposition. Inasmuch as it appeared as though a slow decomposition may be

TABLE IV
MOISTURE LOSS AND REVERSIBILITY OF GLUTEN MEAL AT 60°C

Oven treatment	Interval of drying	Reversible experiment				
		A	B	C	D	E
		Apparent moisture	Reversible moisture value, 96 hours	Adsorption A-B	Decomposition B—80°C	Calculated true moisture A-D
Control	—	8.70	8.80	—	—	—
Vacuum oven 100°C	5	11.26	8.74	2.52	—	—
	16	11.40	8.81	2.59	0.01	11.39
	24	11.42	8.80	2.62	0.00	11.42
	72	11.49	8.87	2.62	0.07	11.42
Vacuum oven 110°C	2	11.28	8.80	2.48	0.00	—
	8	11.47	8.87	2.60	0.07	11.40
	16	11.58	8.92	2.66	0.12	11.46
	24	11.55	9.00	2.55	0.20	11.35
	40	11.64	9.03	2.61	0.23	11.41
Vacuum oven 120°C	2	11.41	8.90	2.51	0.10	11.31
	8	11.59	9.05	2.54	0.25	11.34
	16	11.68	9.08	2.60	0.28	11.40
						Av 11.39

occurring in the air oven at 100°C, the reversibility study was conducted at a lower temperature (air oven, 60°C). Preliminary studies showed that the desorption and adsorption curves for gluten meal were reversible, exhibiting no hysteresis, if the dried feeds were allowed to adsorb as much as 3.0% of water. It was necessary, however, in order to obtain this reversibility, first to moisten both the dried samples and the check, thus putting them on a comparable basis. The vacuum-dried samples and the controls were, therefore, moistened with 8 ml of water and allowed to reach equilibrium in the air oven at 60°C. Considerable weight fluctuations occur at this temperature because of outside humidity changes, and later studies, as shown for the moisture work with corn and corn gluten (papers I and II), eliminated this factor by the use of a constant humidity chamber.

The reversibility data are given in Table IV, from which it can be observed that the average calculated true moisture is 11.39%, which is identical with that obtained by toluene distillation. This agreement, particularly in view of the results obtained with the first sample of gluten meal, which was much more stable, leads again to the conclusion that the toluene distillation method is satisfactory for the determination of the true moisture in gluten meal.

Summary

Moisture studies were conducted on composite samples of gluten meal produced by two companies in the wet milling industry. The results indicate that the true moisture content of gluten meal can be determined. It was found that gluten meal produced by different companies shows variability from the stability standpoint and that a standard oven procedure would be unreliable as a reference method for the determination of the true moisture in this product. Case-hardening and the presence of volatiles affect the oven results. As found previously for corn and corn gluten, the distillation methods are much more reliable for the determination of true moisture. The toluene distillation method, carried to completion, is satisfactory for the determination of the true moisture in gluten meal, and this method has been accepted as the official reference method by the Technical Advisory Committee of the Corn Industries Research Foundation.

The use of the reversibility method for the determination of the true moisture in corn and gluten meal has indicated that this procedure should have a wide application in the determination of the true moisture content in a large variety of products. The method is simple, yields consistent results, and appears to be based on sound premises.

Literature Cited

- Davidson, C. F., and Shorter, S. A.
1930 The dry weight of cotton. *J. Textile Inst.* 21: T165-T178.
- Evans, J. W.
1941 Private communication, unpublished.
- Nelson, O., and Hulett, G. A.
1920 The moisture content of cereals. *Ind. Eng. Chem.* 12: 40-45.
- Pidgeon, L. M., and Maass, O.
1930 The adsorption of water by wood. *J. Am. Chem. Soc.* 52: 1053-1069.
- Urquhart, J. R., and Williams, A. M.
1925 The moisture relation of cotton. II. The absorption and desorption of water by soda-boiled cotton at 25°C. *J. Textile Inst.* 15: T433-T442.
- Van Bemmelen, J. M.
1910 Die Absorption. *Gesammelte Abhandlungen über Kolloide und Absorption.* 196, 214, 232, Dresden, Steinkopff, 548 pages.

THE DETERMINATION OF MOISTURE IN THE WET MILLING INDUSTRY. V. CORN GLUTEN FEED, UNSWEETENED AND SWEETENED

L. SAIR¹ and W. R. FETZER²

In summarizing the investigations reported in this series of papers the Technical Advisory Committee of the Corn Industries Research Foundation clearly indicated that they were not interested in empirical moisture methods. They stated that the objective would be successfully accomplished only if methods were devised which would determine the true moisture content of each product. Later, if desired, the work could be utilized in a more practical form by the calibration of simpler moisture methods against the reference procedures.

It was recognized at the inception of these studies that all former investigations had led to the conclusion that only empirical moisture methods were tenable, since "free water" could not be differentiated from "bound water" and from "water of constitution." This belief suggested that water is held by cereals with varying degrees of tenacity and that a portion of it is so tightly bound that its removal cannot be effected without decomposing the material under test. In view of this situation the moisture method has been considered as a tool which will yield relative rather than absolute moisture results.

Moisture studies with corn, corn gluten, steep water, hulls, and gluten meal have led to conclusions contrary to those previously held regarding moisture in cereals. The moisture problem for corn and its feed products has been approached by several experimental methods not hitherto applied to cereals and it was found that the true moisture content of each product could be determined, and that there is a clear differentiation between free and adsorbed water and water of constitution.

¹ Research Fellow, Corn Industries Research Foundation.

² Chief Chemist, U. S. Starch and Refining Co., Granite City, Illinois.

tion. The results indicated that there is no need to rely on empirical methods for the determination of moisture. Studies with corn (paper No. I) showed that the present official moisture methods underestimate the moisture in corn by approximately 2.0%; in other words, 100 pounds of corn contain 2 pounds less dry substance than formerly thought. These results offer an explanation for the unaccountable loss of 2% to 3%, which in spite of extensive studies could not be accounted for previously in the wet milling industry. Studies with steep water, hulls, and corn gluten (paper No. II) indicated that the present procedures were too empirical. The current methods in use for steep water overestimated the moisture by 2%-4%. Vacuum drying of corn gluten resulted in a marked loss of volatiles. Oven procedures in general yielded only empirical values. Similarly, the practice of limiting the toluene distillation procedure to 1 to 2 hours consistently underestimated the moisture in products such as corn, corn gluten, and gluten meal.

This paper covers moisture studies dealing with gluten, hydrol, and molasses feeds. Corn gluten feed is produced by kiln drying, usually in a two-step process. In the first step, concentrated steep water is added to previously dried hulls which are then passed through a drier at a rate that will produce a product not completely dry. Additional steep water and corn gluten are added to this product, the mass thoroughly blended, and passed to a second drier which reduces the moisture to trade requirements. Thus a moisture test on this product becomes essentially one for steep water plus manipulation to free moisture encased and sealed in the hulls and gluten by the external layer of steep water. Sweetened feeds are produced by mechanically blending hydrol or molasses with finished feed, followed by subsequent processing.

The moisture studies reported in this series were not conducted in the order reported, but rather in what was later considered a more logical sequence. The first studies were carried out with gluten feed. The outcome of this investigation offered the basis upon which the other studies were conducted. In many respects this product offered more problems than the others. Various factors which enter into the moisture test, such as case-hardening, instability, presence of volatiles, and variability of the feeds themselves, play a pronounced role in the determination of moisture in gluten feed. The feed is compounded from both feed products and an unstable sirup (steep water), and as a result the determination of moisture in this product includes all the difficulties attendant on its estimation in an unstable sirup combined with the problem of a feed product. Moreover, in gluten feed the problem is further complicated by the fact that the steep water is

dried upon the hulls in kilns during which time it hardens into a resinous coat, thus entrapping the enclosed water.

The unsuitability of present existing official methods for the determination of moisture in gluten feed can be noted from the results given below. Inasmuch as the feed is composed of both a sirup and a feed, the methods used included those given under grain and stock feeds and also under sugars.

Method—A O A. C.	Apparent moisture %
<i>Grain and stock feed:</i>	
Drying over P_2O_5 in <i>vacuo</i> at 40°C (800 hrs) ¹ .	9.09
Vacuum oven—100°C—5 hrs ²	11.10
Vacuum oven—100°C—24 hrs.	12.80
Toluene distillation—1 hr ³	11.10
Toluene distillation—10 hrs.	12.25
Air oven—135°C—1 hr.	14.35
<i>Sugar and sugar products:</i>	
Air oven—100°C—10 hrs	11.60
Vacuum oven—80°C—16 hrs ⁴	9.76

¹ The official method uses concentrated sulfuric acid in a vacuum desiccator at room temperature.

² The official method states that the product should be heated for 5 hrs or longer until constancy is reached.

³ The official method states that the distillation is usually complete within 1 hour.

⁴ The official method states that a temperature of 70°C should be used and that weighings should be made at 1-hr intervals after the first 2-hr period. Constancy was not reached in the above determination.

The discrepancies shown above demonstrate the present empirical status of the moisture determination. They also indicate the undesirability of the inclusion of a large variety of products under one general procedure. Gluten feed is considered as a grain or stock feed. Yet obviously, all the official methods, or possibly none, are suited to the determination of moisture in this product. Grain and stock feeds differ markedly in their properties and behavior. Because of their nature and mode of preparation, the feeds produced in the brewing industry, the milling industry, the distilling industry, the wet milling industry, and others will exhibit differences that must be accounted for in an official moisture procedure. If the above official methods had been applied to gluten meal, the differences found would have been much smaller than with gluten feed. Yet, all these feed products are considered as similar from the moisture standpoint. Corn oil meal is the ground expeller cake obtained from the expression of corn oil from dry corn germs under high pressure. During this process, some moisture is firmly fixed in the meal and can be removed only in a moisture determination by drastic treatment. This procedure will be covered in the next paper of this series. The results of these investigations indicate that the present official methods do not account for the variability that exists in different feed products.

Plan of investigation: The initial plan involved the application of distillation and De Bruyn procedures. Later, in view of the results of

data, various modifications were introduced which will be dealt with under the appropriate headings. The experimental methods originally applied were identical to those described for corn (paper I). A study was first made on a single composite sample of feed ground to pass a 20-mesh sieve. All samples were placed in 4-ounce screw-top bottles which were stored in the refrigerator until required. Routine analysis of the first sample of gluten feed used is given below:

Moisture	11.19%
Crude protein (N \times 6.25)	25.2%
Ether extract	3.7%
Crude fiber	7.6%
Ash	8.6%
NFE (by difference)	43.7%
Acidity as hydrochloric acid	1.33%
pH	5.2%

Ten g of feed in 100 ml of water was allowed to stand for 30 minutes before the determination by glass electrode.

Moisture results: The apparent moisture values obtained by drying the feed at various temperatures in De Bruyns, and also by distillation with benzene, toluene, and xylene are shown in Figures 1 and 2 and in Table I. The feed sample used for this study was similar to that used for the comparative study by the A. O. A. C. methods. The De Bruyn procedure involves the drying of the feed

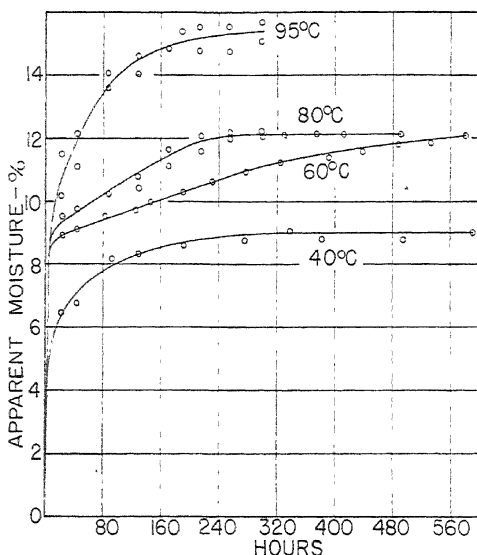


Fig. 1. Time-temperature drying curves for gluten feed—De Bruyn apparatus.

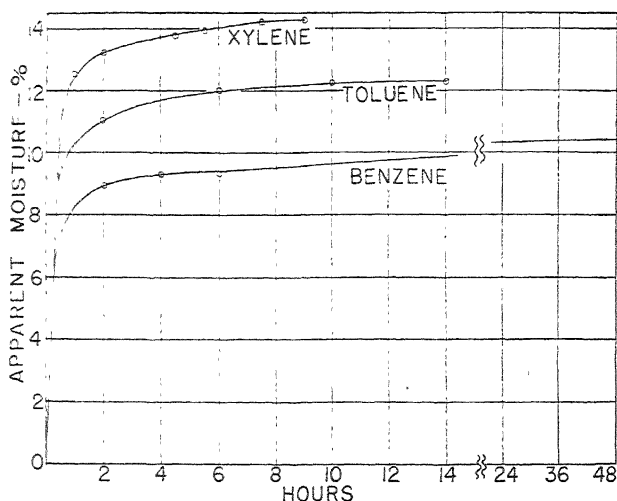


Fig. 2. Moisture of gluten feeds by distillation method.

TABLE I
THE MOISTURE IN GLUTEN FEED

Moisture method	Temperature	Period of drying	Apparent moisture	
			Range during the interval of drying	Final value
De Bruyn	°C	hrs	°C	°C
	40	280-600	8.80-9.00	9.00
	60	280-600	11.00-12.05	12.05
	80	240-480	12.15-12.15	12.15
Distillation	95	160-280	14.80-15.34	15.34
	Benzene—bp 80°C	36-48	10.33-10.33	10.33
	Toluene—bp 110°C	6-14	12.00-12.25	12.25
	Xylene—bp 140°C	5-7	14.00-14.30	14.30

* Weighings were made at regular intervals during the drying period.

in a specially designed apparatus previously described, which permits the drying of the material under high vacuum over P_2O_5 . The flask containing the feed was placed in an insulated box containing the desired number of electric lights, while the flask containing the P_2O_5 was placed in a trough of running water. The temperatures were maintained within 3°C.

As shown in Table I, the apparent moisture of the feed varies from 9.00% to 15.34%. The high values obtained with the De Bruyn at 95°C (15.34%) undoubtedly included losses due to decomposition

as evidenced by the marked discoloration of the feed and the P_2O_5 . Similarly, xylene distillation caused a marked charred appearance in the feed. It will be noted that the toluene distillation values agree reasonably well with the results obtained by drying the feeds in the De Bruyn at 60° and 80°C. Only a slight color formation occurred to the feeds dried in the De Bruyn and a somewhat more marked color occurred to the feed distilled with toluene. In view of this close agreement, it was first thought that possibly the true moisture content of the feed was in the region of 12.0%. The low value in the De Bruyn at 40°C (9.00%) was attributed to severe case-hardening. However, further work indicated that the agreement between the toluene distillation method and the De Bruyn at 60° and 80°C was quite fortuitous, or if not at least caused by the decomposition of some specific fraction of the feed. This was proven by the following experiments.

Use of Filter Aid in the Determination of Moisture in Gluten Feed

Work with steep water (paper II) indicated the necessity of dispersing the material in Hyflo Filter Aid prior to drying. This technique was applied to gluten feed, and by so doing the kiln-dried steep-water solids contained on the hulls were dispersed on the Filter Aid. Ten to 15 g of gluten feed was weighed into 50-ml beakers, to which approximately 20 ml of H_2O was added. The mixture was maintained at a temperature of 40°C for 2 hours, after which it was quantitatively transferred to previously dried De Bruyns containing Filter Aid. The De Bruyns were treated as described previously for steep water. The feeds so treated were then dried at 40° and 60°C along with control samples not subjected to the Filter Aid treatment. The results are shown in Figure 3 and tabulated in Table II, from which it can be observed that the Filter Aid treatment greatly facilitates the removal of the water.

When the softened moist feed was first dispersed on Filter Aid, it can be noted that at 40°C the apparent moisture value increased from 9.00% to 10.00%. The effect of case-hardening is very pronounced with gluten feed, and if not recognized a false equilibrium is obtained. The drying of gluten feed alone at 60°C resulted in a gradual weight loss over the total drying period until a value of 12.05% was reached. No indication of a break in the drying curve was obtained. However, if the feed is first softened by water and then dispersed on Filter Aid, a ready weight loss occurs during the first 90 hours of drying, after which complete constancy is reached for over a 100-hour drying period. Further heating causes an additional weight loss. It will be noted that at 60°C the feed mixed with Filter

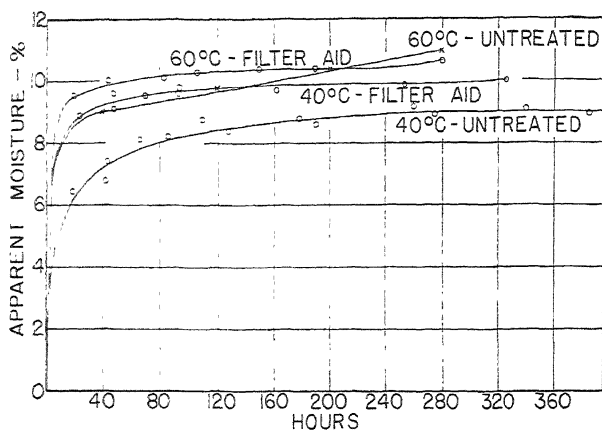


Fig. 3 The effect of Filter Aid dispersion on the determination of moisture in gluten feed.

TABLE II
USE OF FILTER AID IN THE DETERMINATION OF MOISTURE IN GLUTEN FEED

De Bruyns	Period of drying hr.	Apparent moisture	
		Gluten feed alone— range during period of drying	Gluten feed plus Filter Aid— range during period of drying
40°C	160-400	8.68-9.00	9.85-10.00
60°C	90-200	9.50-10.40	10.37-10.37

Continued heating resulted in a continued gradual weight loss with no evidence of constancy until an apparent moisture value of 12.05% was reached

Aid reached constancy at 10.37% within 90 hours, whereas it required 200 hours for the untreated feed to reach the same value. The results suggest that a slow decomposition occurs at this temperature, and that the prolongation in drying necessary for the untreated feed results in such a slow loss of moisture that it cannot be distinguished from the decomposition loss that later occurs. With Filter Aid, however, the moisture is lost so readily that a constancy period can be obtained before decomposition sets in. In the light of these data, it appeared that the moisture range of 10.3%-10.4% was of significance. It will be recalled that the value obtained at constancy by benzene distillation was 10.33%.

Oven Data with Gluten Feed

Further studies by the use of oven methods lent strong support in the determination of the true moisture content of the gluten feed. The feed was dried *in vacuo* at 80° and 100°C and also in the air oven.

at 100°C. The data are shown in Figure 4. It is of interest to note that the air and vacuum oven methods at 100°C yielded essentially similar results. The feed showed a continued weight loss over the total 27-hour drying period and an apparent moisture value of 13.0%

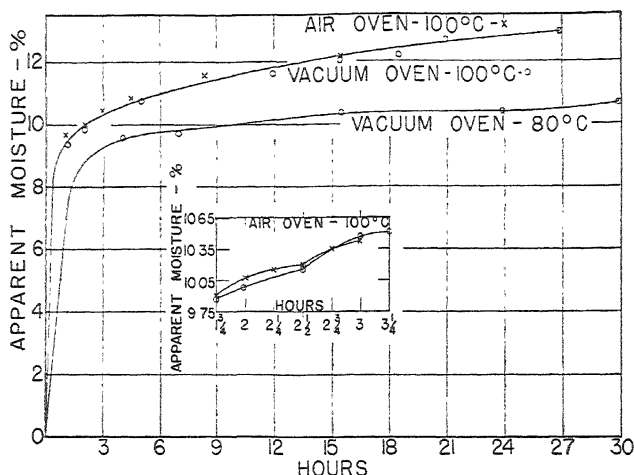


Fig. 4. Oven drying of gluten feed.

was reached. At 80°C *in vacuo* the results appeared to be of more significance. A period of essential constancy was obtained after 16 hours of drying, followed by a more marked weight loss. This is shown in Table III.

TABLE III
VACUUM DRYING OF GLUTEN FEED AT 80°C

Length of drying	Apparent moisture Duplicates	Average	Weight loss per g of feed per hr
<i>hrs</i>	<i>%</i>	<i>%</i>	<i>mg</i>
4	9.55-9.53	9.54	23.7
7	9.78-9.74	9.76	0.73
16	10.38-10.32	10.35	0.64
24	10.42-10.44	10.43	0.10
30	10.80-10.69	10.75	0.54

After 16 hours of drying, a distinct break occurred in the vacuum oven drying curve at 80°C and an additional period of 8 hours resulted in only a slight weight loss. The apparent moisture range during this period was 10.35%-10.43%, which is in agreement with the De Bruyn at 60°C where constancy was reached for 100 hours at 10.37%, and with the constant benzene distillation value of 10.33%.

More Detailed Study of the 100°C Air Oven Curve

The drying of the gluten feed at 100°C in both air and vacuum at hourly intervals indicated no breaks in the drying curve in the region of 10.40%. Later studies indicated that a break actually does occur in this region if samples are removed at 15-minute intervals through this critical moisture range. The results of two separate tests are shown in Figure 4 (inset) where it will be noted that a distinct break occurs when the apparent moisture reaches 10.2%. Reference to the curve will show that if these samples had been dried for half-hour intervals instead of 15-minute intervals, the break would probably have been overlooked. It is believed that this abrupt increase in loss after 2½ hours indicates the inception of decomposition.

Discussion of the Results Obtained with the First Sample of Gluten Feed

Three different moisture methods yielded essentially the same moisture value for the first sample of gluten feed studied. In two cases, complete constancy was reached for a considerable period, whereas in the third (vacuum oven 80°C) the constancy was not altogether complete and was of short duration.

Method	Apparent moisture %
Benzene distillation	10.33
Vacuum oven (80°C—20 hrs)	10.39
De Bruyn (60°C—Filter Aid)	10.37

It was also found that a break occurs in the drying curve at 100°C at the 10.20% level. By drying the feed in admixture with Filter Aid at 40°C, a value of 10.0% was obtained.

The above studies were conducted prior to the investigation carried out with steep water, hulls, and corn gluten, which are the components used in the preparation of gluten feed. These three components have been dealt with in a separate paper of this series (paper III), from which it was indicated that steep water is markedly unstable and that drying at higher temperatures or toluene distillation results in a marked decomposition. The benzene distillation method was found suitable for the determination of moisture in steep water and has been accepted as the official reference method by the T. A. C. of the C. I. R. F. The results for gluten feed given above indicated that the benzene distillation method should prove equally suitable for gluten feed. Studies with other feed samples indicated this belief to be valid. The results obtained are given in the following sections

Further Moisture Studies with Gluten Feed

In view of the agreement obtained by the use of the benzene distillation method, the Filter Aid De Bruyn method at 60°C, and the vacuum oven method at 80°C for the first sample of gluten feed studied, the work was repeated with three additional commercial samples ground to pass a 20-mesh sieve. The results are shown in Table IV. An immiscible solvent boiling at a temperature lower than benzene was also included, as well as one of the moisture methods used in the corn industry.

TABLE IV
THE MOISTURE CONTENT OF GLUTEN FEED

Moisture method	Period of drying	Sample I	Sample II	Sample III
	<i>hrs</i>	<i>%</i>	<i>%</i>	<i>%</i>
Benzene ¹ distillation, 30-g samples	17	8.13 ²	7.73	8.33
	20	8.20	8.07	8.97
	25	8.27	8.07	9.00
	41	8.33	8.07	9.07
	48	8.33	—	9.07
	60	—	—	9.07
Vacuum oven, 80°C	16	8.52	7.99	9.08
	24	8.71	8.16	9.30
Average	—	8.62	8.08	9.19
De Bruyn, 60°C, dispersed on Filter Aid	60	8.05	7.82	9.01
	82	8.50	7.94	9.09
	104	8.57	8.04	9.13
	126	8.66	8.10	9.19
	148	8.74	8.15	9.25
	170	8.87	8.17	9.29
Average	—	8.67	8.08	9.19
Skelly-Solve (petroleum ether bp 55°–70°C)	70	6.96	6.66	8.27
Union Starch method	—	9.38	8.66	9.74

¹ A correction of 0.02 ml is required when using benzene.

² Intermediate values were taken during the course of the distillation and are only approximate.

The three feeds showed some variation from the first composite sample used. In no case was absolute constancy reached with the De Bruyns at 60°C, the feeds exhibiting a continued weight loss of 0.03% to 0.10% daily after the first 82-hour drying periods. As a result, an approximation is necessary in determining the moisture content by the De Bruyn method. This was done by averaging the values between the 82- and 170-hour drying period. These results

are then in agreement for samples No. 2 and No. 3, with the direct moisture values obtained by benzene distillation and also with the average of the 16- and 24-hour values *in vacuo* at 80°C. The distillation results for sample No. 1 were low as compared to both the De Bruyn and vacuum oven at 80°C. Later work has suggested that this may have been caused by very severe case-hardening. It will be noted that Skelly-Solve, which boils at 55°–70°C, is quite inadequate for the removal of the total water—underestimating the moisture by 0.9%–1.7%. The commercial method, as compared to the benzene distillation values, overestimates the moisture by 0.6%–1.0%.

In view of the De Bruyn results at 60°C, which yielded no constant value for any period, thus suggesting a slow decomposition, the work was repeated with four additional samples, but in this case the De Bruyn tests were conducted at 50°C rather than 60°C. By so doing it will be noted from Table V that a definite plateau is obtained which is in agreement with the benzene distillation results.

TABLE V
THE MOISTURE CONTENT OF GLUTEN FEED

Sample No.	Benzene distillation (constancy—42 hrs.)	De Bruyn—50°C		
		Drying period of constancy ¹	Moisture range	Final moisture value (average)
	%	hrs	%	%
1	7.60	370–505	7.53–7.55	7.55
2	9.80	370–525	9.71–9.82	9.78
3	9.90	370–620	9.80–9.92	9.87
4	9.10	460–620	9.12–9.17	9.14

¹ Weighings made every 48 hours. The vacuums were maintained at less than 0.1 mm.

The results in Table V added further confirmation to the previous data. In one case, water is determined directly, and in the second, the feed is dried to constancy in high vacuum over P_2O_5 after dispersion on Filter Aid, at the maximum temperature allowable. The results agree, as shown above, within 0.1% for the four feeds tested. These data, in conjunction with the results previously reported for steep water and hulls (paper III), indicated that either the benzene distillation method or the De Bruyn procedure of softening the feed and dispersing it on Filter Aid before drying to constancy over P_2O_5 at 50°C would prove equally suitable as reference methods for the determination of moisture in gluten feed. Further work indicated, however, that the De Bruyn procedure would be quite unsuitable since in the feeds produced by some of the members of the industry, a considerable quantity of volatiles are present, which volatilize in the De Bruyn and, as a consequence, yield moisture values wh

are too high. This was found to be true also for hydrol and molasses feeds. Typical data obtained for the feeds produced by several members of the industry are given in Table VI.

TABLE VI
THE MOISTURE OF MOLASSES, HYDROL, AND GLUTEN FEEDS

Product	Benzene distillation	Moisture method De Bruyns at 50°C. Filter Aid 400-700 hrs	Air oven, 100°C 3 hrs
Gluten feed—No. 1	7.60	8.10	7.69
Gluten feed—No. 2	7.20	7.65	7.26
Gluten feed—No. 3	9.94	10.84	10.05
Gluten feed—No. 4	10.58	11.25 ¹	10.40
Gluten feed—No. 5	11.18	11.25	11.05
Hydrol feed	8.03	8.13	8.12
Sweetened feed (15%)	9.76	9.91	9.70
Sweetened feed (20%)	8.76	9.40	9.20

Volatiles equivalent to 0.25% were isolated from this sample by the use of dry ice

It will be noted that the three-hour air-oven method at 100°C. which was previously found to be in fair agreement with the benzene distillation, is also in agreement for the gluten feed samples given in Table VI. The De Bruyn procedure of drying at 50°C, however, yields results which are 0.10% to 0.90% higher than the benzene distillation values. That this discrepancy is not largely due to severe case-hardening which prevents the liberation of moisture by benzene distillation was evident by the presence of volatiles which were noted in the connecting tube between the feed sample and the P₂O₅ flask. In one case, by a relatively crude method, 0.25% of volatiles was isolated. This variability in feed is to be expected in view of the method of preparation. Some companies immediately concentrate the steep water, leaving the steeping tanks to the desired Baumé, while others allow the steep water to go through an initial fermentation period. By so doing, they have found that a higher concentration of solids can be obtained in the steep water without tube incrustation. In other cases some of the effluents from the modified starch products are added to the steep water. As a result the variability found with respect to volatiles in the different feeds is to be expected. In view of this variability, the only method considered suitable as a reference procedure for the determination of the true moisture in gluten feed was the benzene distillation procedure. It is recognized that this method may, in isolated cases, yield low values because of unduly severe case-hardening. Further work has shown that in certain cases constancy during distillation is not attained for 70 to 100 hours. However, the results so far obtained have indicated that this error in will be of reasonably small magnitude. In any event the work shows

that no indirect moisture method could prove suitable for products of the nature of gluten, hydrol. and sweetened feeds.

Relation of Storage to Moisture in Gluten Feed

During these studies samples were stored for relatively long periods. In certain cases, to conserve space in the refrigerator, the samples were allowed to stand at room temperature which, during the summer months, ranged from 90° to 100°F. With storage a noticeable darkening in the color of the feed occurred. Studies were conducted to determine whether this color change was accompanied by an actual DS loss in the feed, and whether as a result, the apparent moisture of feed would increase with storage.

Studies have been conducted covering the effect of moisture content, of pH, and of temperature of storage on the keeping qualities of gluten feed. Only that work pertinent to the moisture problem of gluten feed will be dealt with here. The results of a single typical study are given.

Samples of a thoroughly mixed sample of gluten feed (100 g) were placed in one-pound friction-top tins and stored for varying lengths of time at different temperatures. Considerable pressure developed in the tins during storage at the higher temperatures and as a result some leakage occurred (0.2%–0.4%). This loss was prevented in subsequent work by soldering the lids. Upon completion of the storage period, the samples were transferred to the refrigerator until required.

A moisture analysis of the products subjected to the various storage treatments was conducted by the following procedures:

De Bruyn. Filter Aid—50°C: The dry substance present in the sample used for the storage study was determined by drying the feed to constancy, after admixture with Filter Aid, over P_2O_5 at 50°C in De Bruyns. The feed contained 90.54% dry substance (9.46% moisture). The samples stored for the different periods were then dried under similar conditions, and moisture values greater than 9.46% can be attributed to changes caused by the storage treatment. This method gives a measure of the dry substance loss. This study was the first application of the reversibility method (paper IV) to the determination of moisture in feeds or cereals.

Benzene distillation: The moisture content of the feed, as determined by the benzene distillation method, was 9.45%. This value is in agreement with the De Bruyn results, indicating that this particular feed contained no volatiles that will volatilize under the conditions used in the De Bruyns. The moisture contents of the various stored feeds were determined by the benzene distillation procedure. Valu

in excess of 9.45% can be attributed to the formation of water of decomposition. A comparison of the distillation and De Bruyn results shows the relation between water of decomposition and the total dry substance loss.

The results of the storage study are given in Table VII. These results indicate the need of storing gluten feed at low temperatures.

TABLE VII
STORAGE AND MOISTURE OF GLUTEN FEED

Condition of storage	Apparent moisture			
	Benzene distillation—72 hrs		De Bruyn—50°C, 500 hrs	
	Moisture	Water of decomposition	Moisture	Dry substance loss
Control	9.45	—	9.46	—
4 wks at 38°C	9.68	0.23	9.89	0.43
2 wks at 50°C	10.41	0.96	10.42	0.96
4 wks at 50°C	10.60	1.15	10.70	1.24
2 wks at 60°C	10.84	1.39	11.14	1.68

* Constancy in weight during the last 150 hours of drying.

Four weeks of storage at 38°C (100°F) resulted in a dry substance loss of 0.43%, of which 53.5% was due to water of decomposition. Two weeks at 50°C (120°F) caused a dry-substance loss of 0.96%, which could be wholly attributed to decomposition due to the splitting off of water. This decomposition was accompanied by an increasing discoloration of the feed. The results demonstrate the thermolability of gluten feed and show that the major product of decomposition is water. Later work has indicated that 10% to 15% of the total dry-substance loss is attributable to the formation of gas, the nature of which has not been determined.

As pointed out in a previous paper (II) steep water, which is one of the major constituents of gluten feed, is largely composed of nitrogenous hydrolytic products, ash, and reducing sugars. Some ammonia nitrogen is also present. During storage some of these products apparently condense with the splitting off of water. If the feed is stored with a low moisture content (3%), the decomposition is greatly lessened, indicating that in this case insufficient water is present to allow for the movement of the interacting constituents.

The results obtained in the storage study add further confirmation to the use of the benzene distillation method for the determination of moisture in a product such as gluten feed. It is of interest to note the parallelism that occurs between determining the moisture directly and the results obtained by the indirect procedure (De Bruyn).

The data obtained by the use of the benzene distillation method and the De Bruyn method in the study of decomposition of gluten feed suggests that these procedures might have a wide application. They may prove of value in the approach to studies dealing with the interaction of sugars and nitrogenous products, or for that matter to any condensation reaction, resulting in the splitting off of water. The relation of water formation to the total dry substance changes can be determined with precision.

Summary

The Technical Advisory Committee of the C. I. R. F. has accepted the benzene distillation method, carried to completion, as the official reference moisture method for the determination of moisture in corn gluten feed, sweetened and unsweetened.

The results of these investigations suggest that the benzene distillation method should have a wide utility for the determination of moisture in thermolabile products, such as hydrolytic protein mixtures. It should also prove suitable for cereal products such as malt, distiller's and brewer's grain, and other processed products which may be unstable to heat.

THE DETERMINATION OF MOISTURE IN THE WET MILLING INDUSTRY. VI. CORN OIL MEAL

L. SAIR¹ and W. R. FETZER²

This paper on corn oil meal completes the series dealing with the moisture in corn and its feed by-products. The germ floating off in the wet milling process is thoroughly washed, passed through a moisture expeller, and is then dried in kilns to a moisture value approximating 1.0%. It is then preheated to approximately 95°C before passing through an oil expeller which exerts a pressure of approximately 4,000 pounds per square inch in removing the oil. The ground expeller cake is sold under the trade name of corn oil meal.

A moisture study was made on a single composite sample of corn oil meal. The procedures used included distillation methods, De Bruyn method, oven methods, and reversibility studies. The procedure of sampling and the experimental details were similar to those described for corn (No. I of this series). The meal as received from the plant is ground fine and completely passes through a 20-mesh sieve. Seventy percent of the material passes through a 30-mesh

¹ Research Fellow, Corn Industries Research Foundation.

² Chief Chemist, Union Starch and Refining Co., Granite City, Illinois.

sieve. Routine analysis of the corn oil meal used in this study yielded the following results:

Moisture	10.1
Protein (N \times 6.25)	23.5
Ether extract	10.0
Crude fiber	9.2
Ash	2.5
NFE (by difference)	44.7
Acidity as HCl	0.52
pH	4.6

Moisture Results

The moisture results obtained by the use of distillation methods, De Bruyn methods, and by oven methods are given in Table I, which again illustrates the variation in moisture values with different methods. The moisture range varied from 9.94% to 10.69%. The results in Table I show that the obtained moisture values fall roughly into three groupings. The lower moisture range of 9.94% to 10.17% was obtained by drying the meal in an air oven at 100°C for 72 hours (9.94%), by drying in a De Bruyn over P_2O_5 at high vacuum at 60°C for 272 hours (10.17%), by drying in the vacuum oven at 80°C for 120 hours (10.04%), and by benzene distillation (10.12%). The highest value was obtained by vacuum drying at 115°C for 40 hours (10.69%). The intermediate values were obtained by toluene distillation (10.42%), by De Bruyn drying at 90°C (10.36%), and by vacuum drying at 100°C for 40 hours (10.36%) and for 120 hours (10.46%).

TABLE I
MOISTURE OF CORN OIL MEAL BY VARIOUS METHODS

Moisture method	Temperature	Length of drying, showing period of essential constancy	Moisture range during period of essential constancy	Final moisture value	
				Duplicates	Average
De Bruyn	°C	hrs	°C	°C	°C
	60	132-272	10.16-10.17	10.18-10.16	10.17
	90	88-272	10.33-10.37	10.36	10.36
Oven drying	Vacuo 80	40-120	10.02-10.04	10.04-10.05	10.04
	(1) 100	18- 40	10.24-10.36 ¹	10.36-10.35	10.36
	(2) 100	40-120	10.28-10.46 ¹	10.46-10.46	10.46
	115	8- 40	10.41-10.69 ¹	10.68-10.70	10.69
	Air 100	18- 72	9.90- 9.94	9.97- 9.92	9.94
Distillation	Benzene	21- 68	10.14-10.12	10.08-10.16	10.12
	Toluene	21- 45	10.42	10.49-10.29-10.43 10.47	10.42

¹ No period of constancy reached in the vacuum oven at 100° and 115°C.

oil meal prevented the liberation of the total moisture. That this is the case is proved by the reversibility study reported in Table IV.

Table IV shows that vacuum drying at 100°C for 96 hours causes no appreciable decomposition in the corn oil meal, as evidenced by

TABLE IV
FURTHER STUDY OF REVERSIBILITY AND MOISTURE OF CORN OIL MEAL

Drying treatment	Apparent moisture %	Reversible experiment	
		Reversible moisture value	Adsorption %
<i>Vacuo</i> 80°C 68 hrs	9.97	10.14 10.09 Av 10.12	-0.15
<i>Vacuo</i> 80°C 44 hrs 100°C 72 hrs	10.43	10.15 10.16 Av 10.16	0.27
<i>Vacuo</i> 100°C 96 hrs	10.42	10.17 10.12 Av 10.14	0.28

* Samples moistened and re-dried for 48 hrs *in vacuo* at 60°C

the fact that the feed reaches essentially the same reversible moisture value as does the feed dried *in vacuo* at 80°C. It is of interest to note that by moistening the feed dried *in vacuo* at 80°C, prior to redrying at 60°C *in vacuo*, the material lost rather than gained moisture. This result adds further confirmation to the evidence indicating that the low values obtained by vacuum drying at 80°C are attributable to case hardening.

The above results, taken in conjunction with those previously given, demonstrate that values in the lower moisture range—those obtained by benzene distillation, by the air oven at 100°C, by the vacuum oven at 80°C, and by the De Bruyn at 60°C—are lower than the amount of moisture actually present.

A third reversibility experiment was conducted with the samples dried *in vacuo* at 115°C, from which it is evident (Table V) that prolonged drying under those conditions results in the decomposition of the feed.

The meal dried for 2.5 hrs *in vacuo* at 115°C was used as the control sample. This was necessary in view of the severe case-hardening of corn oil meal, which results in moisture values for controls that are lower than would otherwise be obtained. This feature was previously dealt with with 10-mesh corn (II of this series). The results show that the vacuum drying of corn oil meal for 8 hours causes no loss in volatiles or decomposition, as evidenced by the fact that the meal sample is perfectly reversible to the value obtained by

TABLE V
THIRD STUDY OF REVERSIBILITY AND MOISTURE OF CORN OIL MEAL

Drying treatment: <i>vacuo</i> —115°C	Reversible experiment				
	A Apparent moisture	B Reversible moisture value	C Adsorption	D ² Decom- position B—10.00	E Calculated true moisture A-D
2.5	10.31	9.98 10.02 Av 10.00	0.31	—	—
8	10.41	10.00 10.01 Av 10.00	0.41	—	10.41
16	10.50	10.08 10.12 Av 10.10	0.40	0.10	10.40
24	10.58	10.20 10.22 Av 10.21	0.37	0.21	10.37
40	10.69	10.36 10.34 Av 10.35	0.34	0.35	10.34 Av 11.38

¹ Samples redried for 48 hrs in the air oven at 100°C.

² Decomposition includes the loss of volatiles

drying the feed for only 2–5 hours. The results show that an apparent moisture value of 10.41% does not include losses other than water. Beyond this value, however, decomposition does occur as evidenced by the disparity in the reversible values. It will be noted that the average calculated true moisture for this sample of corn oil meal is 10.38%.

The calculated moisture value by the reversibility study (10.38%) is in agreement with the De Bruyn method of drying to constancy at 90°C (10.36%) and with the toluene distillation method (10.42%). Studies with corn (II of this series), with corn gluten (III), and with corn gluten meal (IV) have shown that the toluene distillation procedure yields results in agreement with the reversibility method. The toluene distillation method has been accepted by the Technical Advisory Committee of the C. I. R. F. as the official reference method for the determination of moisture in these products, and the results of this study indicate that the toluene distillation method is also satisfactory for corn oil meal.

Reference to Figure 1 shows that it requires 80 to 90 hours *in vacuo* at 100°C to remove the total moisture from corn oil meal. This is in contrast to other products, which may lose their total moisture in 5 to 20 hours. This result again demonstrates the danger involved attempting to generalize with a specific indirect procedure.

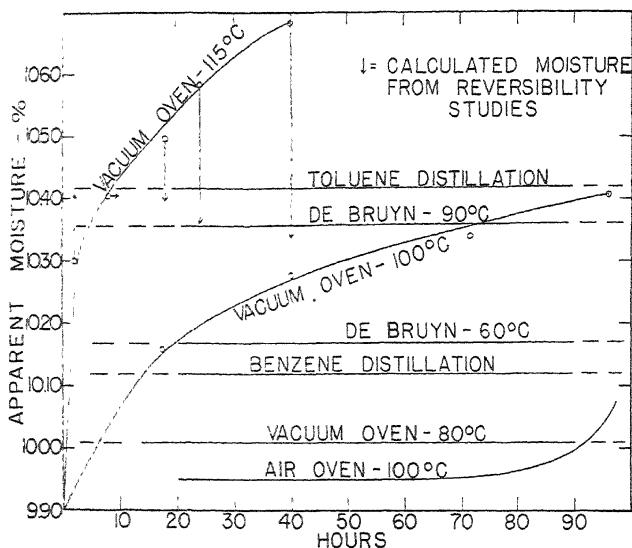


Fig 1 The determination of moisture in corn oil meal by various methods

Conclusion

The results of these investigations with corn and its feed by-products have led to the recommendation of two official reference moisture methods within the wet milling industry. Both methods determine moisture directly. One method (toluene distillation) is to be used with the stable products, which include corn, hulls, corn gluten, gluten meal, and corn oil meal. The second (benzene distillation) is used for the unstable products, which include gluten feed, steep water, and sweetened feeds prepared by the addition of either hydrol or molasses. These two procedures must be carried to completion, since otherwise values are obtained which must be termed empirical. The feeds must be ground to pass at least a 20-mesh sieve. With steep water it is necessary first to disperse the material on Hyflo Filter Aid. If the necessary recognition is given to the various factors which enter into a moisture determination, these two recommended procedures not only give true moisture values for the products produced in the wet milling industry, but we believe they could be extended to similar moisture determinations in other industries.

The usual indirect moisture methods can only yield relative results. Cereals and feeds differ in the amount of volatiles present, in the tenacity with which water is held, and even in stability. For rap

moisture tests the oven procedures undoubtedly are of value, but they cannot be considered as absolute methods, and as such should not be termed official methods. The official desiccator method, which makes use of sulfuric acid as a desiccant, does not recognize the very important factor of case-hardening in feeds and cereals. Moreover, it is difficult to maintain a suitable vacuum in the desiccators. The results of these investigations would indicate that this procedure will only yield relative results.

The results of these investigations indicate that the two distillation procedures, the benzene and toluene distillation methods carried to completion, are the only present satisfactory distillation methods for the determination of the true moisture in cereals and feeds.

Acknowledgments

This series of papers dealing with the determination of moisture in the wet milling industry is based on nine progress reports made to the Technical Advisory Committee of the Corn Industries Research Foundation, comprised as follows:

Norman F. Kennedy, Director of Research
H. H. Schopmeyer, American Maize Products Company
George S. Bratton, Anheuser-Busch, Inc.
R. L. Wagner, Clinton Company
W. B. Newkirk, Corn Products Refining Company
R. L. Albright, The Hubinger Company
L. M. Thomas, The Keefer Starch Company
A. A. Holden, National Starch Products, Inc.
J. M. Widmer, Penick & Ford, Ltd., Inc.
Howard File, A. E. Staley Manufacturing Company
W. R. Fetzer, Union Starch and Refining Company

The authors' thanks are due to the Technical Advisory Committee for their helpful criticism and suggestions during the course of this investigation and their permission to publish this work. We wish to acknowledge the helpful studies on 20-mesh corn carried out by Messrs. Barry Scallet and Otto Eble of the Anheuser-Busch Laboratories, which added additional confirmation to our work.

The Research Fellow wishes to thank the Union Starch and Refining Company for laboratory facilities and the staff for their helpful co-operation, particularly Dr. J. W. Evans for criticism and assistance in the preparation of these papers and Mrs. F. Lewis for the analyses of various feed products.

APPENDIX

OFFICIAL REFERENCE METHOD OF THE CORN INDUSTRIES RESEARCH FOUNDATION FOR THE DETERMINATION OF MOISTURE IN CORN

Toluene Distillation Method

APPARATUS

Mill: Wiley Laboratory Mill: Intermediate Model No. 4267 P, with necessary accessories. Fisher Scientific Co. (1 extra 20-mesh sieve). The mill as received should be changed as follows: Drill a 1 1/4" hole in the lid of the hopper. Prepare a plunger of hard wood 6" long, 2" of the length being 1 1/2" in diameter and the remainder a little less than 1 1/4".

Weighing bottles: 60 X 40 mm Pyrex, standard taper 40/12; stopper also used with distillation flasks described below.

Distillation apparatus: Blueprints covering the apparatus used are on file with the S.G.A. Company or a blueprint may be obtained from the Corn Industries Research Foundation, 5 East 45th St., New York City. The individual pieces are as follows:

Erlenmeyer flasks: 250-ml Pyrex standard taper 40/50.

Distillation traps: Pyrex standard taper 40/50—24/40, 5-ml capacity graduated in 1, 10 ml. The connecting tube between the 40/50 joint and the trap proper should be insulated. A satisfactory method is to wrap this tube with at least three layers of electricians' tape, overlapping each turn, followed by two coats of heavy shellac.

Condensers: Pyrex, straight tube, similar to West type, 40-cm jacket, water tubes on same side, 24/40 standard taper water-cooled joint, lower end: fitted with drip tip with ring seal, the tip being opposite to water tube. The top of the condenser is an extension of the inner tube, 10 cm long.

Stainless steel baths: Dia 4", depth 4", home-made.

REAGENTS

Asbestos: Medium fiber, acid washed.

Toluene: Reagent grade.

PREPARATION OF APPARATUS

1. *Distillation flasks and asbestos:* Distillation flasks containing 5 to 8 g of asbestos are dried overnight in an air oven at 100°C. The flasks, which are first stoppered, are cooled in a desiccator containing calcium carbide or calcium chloride. To prevent the freezing of the glass stopper a thin strip of paper is inserted between it and the flask.

2. *Cleaning of traps and condensers:* The traps and condensers must be scrupulously clean. The traps are first thoroughly scrubbed with water and cleanser. A small narrow brush is necessary to reach to the bottom of the trap. At the end of this stage no droplets of water should stick to the inner sides of the traps. Hot fresh cleaning solution is then added and the traps are left for periods ranging from two hours to overnight. Glass stoppers standard taper 40/50 are much more satisfactory than rubber stoppers for closure of the standard taper 40/50 end of the trap. The traps are then thoroughly rinsed with distilled water and are refilled with a 1% solution of caustic soda. After 15 to 30 minutes the traps are again very thoroughly rinsed. Drying of the traps has been carried out by inverting them on a towel, and leaving them upright overnight. A second towel is placed above to prevent dust accumulation. If desired the drying operation can be hastened by the use of an oven. The condensers are cleaned in the same manner except that the water-cleanser treatment is not used.

PREPARATION OF SAMPLE

1. *The sampling of corn:* Every care must be taken to insure uniformity in the sample of corn under test. Whole corn kernels of uneven moisture require considerable time to reach equilibrium, much more so than when the material is in the ground state. It is necessary, therefore, if only a small sample is available, to shake the corn for some time in a bottle filled to one-third capacity. If a large sample is used, then some form of commercial sampler such as the Boerner sampler should be used.

2. *The moisture of corn:* If the moisture content of the corn is too high to be directly ground in the Wiley, it is necessary first to pre-dry the material.

Fill previously tared moisture dishes (with covers) with the damp grain and weigh. Place the dishes (cover removed) in a warm, well ventilated place (preferably on top of heated oven) protected from dust so that the grain will dry reasonably fast and reach an approximate air-dry condition in from 14 to 24 hours. Weigh the dishes and calculate the percentage of moisture lost in air-drying. Calculate the percentage of total moisture in original sample as follows:

Total moisture	= $A + (100 - A)B / 100$,
where total moisture	= % total moisture
A	= % moisture lost in air-drying
B	= % moisture in air-drying sample as determined by toluene distillation.

With corn of usual moisture content (10% to 20% or possibly inter-
mediate) this pre-drying operation is unnecessary; 20 to 30 g of corn,

depending on its moisture content, is weighed in a glass-stoppered weighing bottle.

MILL PROCEDURE

The weighed corn is ground quantitatively in a small Wiley mill. The Wiley mill is of the shearing type and during grinding very little heating occurs. The procedure of grinding is as follows: The corn is transferred to the closed hopper, and the lid is replaced. The 20-mesh sieve, connected to a glass receiving bottle, is fixed in position. The small glass plate is tightly screwed to the face of the mill. A clean sheet of paper is placed under the receiving bottle in order that any falling particles may be recovered.

After the motor has been turned on, the wooden plug which seats the hopper is raised. The raising and lowering of this plug facilitates the steady flow of the kernels. No kernels should be allowed to enter the mill unless the motor is on, since otherwise the mill will become plugged.

Upon completion of the grinding, the mill is carefully disassembled. Any dust in the hopper is brushed into the mill with a camel's hair brush. The dust accumulating on the rim of the glass plate is brushed on to the sheet of paper. The mill is then brushed completely, care being taken to insure that all particles between the knife plates are removed. The ground material is retransferred to the weighing bottle which contained the whole corn. The moisture loss during grinding is determined. This value will vary with the moisture of the corn, the outside temperature, etc. Typical results obtained are as follows:

Sample	Moisture of corn	Grinding loss, %, duplicates
1	13.66	0.38; 0.37
2	14.18	0.30; 0.29
3	16.14	0.44; 0.45
4	17.58	0.57; 0.60

DETERMINATION OF MOISTURE

The ground corn is transferred to the distillation flask and is washed out with increments of toluene to insure a complete transfer. Approximately 75 ml of toluene is added. The apparatus is then connected.

TREATMENT OF JOINTS OF THE ASSEMBLY

A few drops of xylene are used to seal the glass joints between the flask, trap, and condenser. A few stiff turns seal the joints tightly. As a precautionary measure it has been found advisable also to seal the top connection between the trap and condenser with a few turns of electrician's tape. The traps are filled with toluene before assembly.

With these precautions, distillation can be continued for days without any appreciable leakage.

CONDENSERS

A steady flow of water is at all times maintained during the distillation. An inverted glass test tube, which fits snugly on the top of the condenser, is used to reduce to a minimum the movement of air to and from the condenser.

OIL BATH

The oil baths are small stainless steel (or copper) containers with a screen insert one inch from the bottom, filled with corn oil. To prevent undue heat loss during distillation the baths have been insulated. An outer metal shield covered with asbestos fits snugly (within $\frac{1}{8}$ "') around the oil baths. The baths are also covered during distillation with cardboard containing an opening for the neck of the flasks.

DISTILLATION

After the apparatus is completely assembled the heaters are turned on. Distillation is started quite slowly (1 to 2 drops per second) but can be hastened after the first half-hour period to about 2 to 4 drops per second. The distillation is so conducted that the distillate is condensed in the bottom two inches of the condenser. After the first hour, and then from time to time, a few milliliters of toluene is poured into the top of the condenser to wash down any droplets that may have formed inside the tube. If the apparatus is properly cleaned it will be found that a clean, round meniscus is formed and that no water or only a trace sticks to the sides of the trap. Distillation should be continued for 48 hours.

TREATMENT OF THE TRAPS

Before the apparatus is disassembled, the condenser should be thoroughly washed down with toluene. If properly cleaned, no droplets of water should stick to the inside tube, and the condenser tube surface, if examined under the light, should be completely free of contamination.

A small piece of clean rubber tubing is attached to the end of a stiff wire and this is used for thoroughly scrubbing down the sides of the traps so that any occluded water may settle. The traps are then placed in water at 68°F to reach equilibrium (1 to 2 hrs) before reading.

CORRECTION FACTOR

A value of 0.03 ml is added to the obtained water value to compensate for the water that remains in the toluene.

FURTHER DISTILLATION

Since in some cases it has been found that 48 hours of distillation is insufficient it is advisable to reassemble the apparatus and continue the distillation for another period. Experience may indicate that this is unnecessary under most circumstances. The course of the distillation can be followed closely by taking readings during the distillation.

CALCULATIONS

Sample wt of corn.	25.4995 g
Wt ground corn.	25.3861 g
Moisture loss during grinding.	0.1134 g
Ml of water obtained.	3.99
Ml of water plus correction (0.03).	4.02
Total moisture (4.02 + 0.1134).	4.1334 g
Moisture, %.	16.21

**OFFICIAL REFERENCE METHOD OF THE CORN INDUSTRIES
RESEARCH FOUNDATION FOR THE DETERMINATION
OF MOISTURE IN CORN GLUTEN MEAL
AND CORN OIL MEAL**

*Toluene Distillation Method**APPARATUS AND REAGENTS*

Similar to those described for corn.

PREPARATION OF APPARATUS

Similar to that described for corn.

PREPARATION OF SAMPLE

Similar to that described for corn.

MILL PROCEDURE

Commercial corn oil meal is usually ground to pass 20 mesh, and further laboratory treatment is unnecessary. Gluten meal must be quantitatively ground, as described for corn, since a portion of the gluten meal dries into hard, horny pellets of varying sizes.

DETERMINATION OF MOISTURE

The procedure used is similar to that described for corn.



OFFICIAL REFERENCE METHOD OF THE CORN INDUSTRIES
RESEARCH FOUNDATION FOR THE DETERMINATION
OF MOISTURE IN CORN GLUTEN FEED
AND SWEETENED FEEDS

Benzene Distillation Method

APPARATUS

Similar to that described for corn.

REAGENT

Benzene, reagent grade.

PREPARATION OF APPARATUS

Similar to that described for corn.

PREPARATION OF SAMPLE

Similar to that described for corn.

MILL PROCEDURE

The gluten feed should be ground quantitatively as described for corn.

DETERMINATION OF MOISTURE

The procedure used is similar to that described for corn. A correction factor of 0.02 ml is added to the obtained water value rather than 0.03 ml, which is used for toluene. Forty-eight hours of distillation is the minimum time required for the complete removal of water, and in certain cases distillation must be prolonged for periods ranging up to 80 hours.

OFFICIAL REFERENCE METHOD FOR THE DETERMINATION
OF MOISTURE IN STEEP WATER

Benzene Distillation Method

APPARATUS

Distillation apparatus: Similar to that described for corn.

Test tubes: Pyrex 100 × 13 mm. Used as pestles for incorporating the steep water into the Filter Aid.

REAGENTS

Benzene: Reagent grade.

Filter Aid: Hyflo, Johns Manville, New York. A large quantity of Filter Aid is washed by percolation with distilled water that has been

slightly acidulated with hydrochloric acid. This treatment is continued until the effluent is acid to litmus. Washing with distilled water follows until the effluent is essentially neutral, and the Filter Aid is then air-dried. A quantity, usually a quart, is transferred to an air oven at 105°C and kept for use.

PREPARATION OF SAMPLE

Steep water deposits suspended material on standing and the contents should be well shaken before being sampled.

PROCEDURE

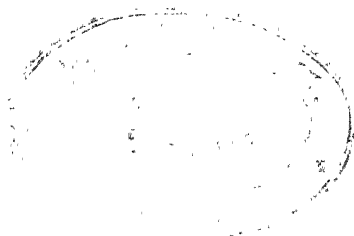
Distillation flasks containing 15–20 g of Filter Aid and a test tube are dried to constancy (24 hours) *in vacuo* at 100°C. The flasks on removal from the oven are immediately stoppered (standard taper 40, 12) and allowed to cool over P_2O_5 . A thin strip of paper is inserted between the stopper and flask to prevent the freezing of the stopper. The flask is then weighed, using a tare weight, and 8 to 12 g of steep water, depending on its Baumé, is quickly transferred by means of a pipette with an open end to the flask, which is then re-weighed. The steep water is rapidly incorporated into the Filter Aid by inserting a glass rod into the test tube and mixing the contents. Seventy-five milliliters of benzene is then added and the mixture is given a further rapid mixing. The flask is then attached to the trap and condenser, the trap is filled with solvent, and distillation is conducted in the manner described for corn. The water is usually completely removed within 24 hours. A correction factor of 0.02 ml is added to the obtained water.

Memorial

TO

CARL LUCAS ALSBERG

1877-1940





CARL LUCAS ALSBERG, 1877-1940

CEREAL CHEMISTRY

VOL. XIX

NOVEMBER, 1942

No. 6

CARL LUCAS ALSBERG

CARL LUCAS ALSBERG died suddenly on October 31, 1940, at Berkeley, California, in his 64th year. Students of the natural and social sciences alike, all over the world, have lost a farseeing leader, a wise counselor, and an energetic and co-operative fellow-worker.

Alsberg was born in New York City in 1877, the eldest of four children of German-Jewish stock in a middle-class family of intellectually liberal tradition. The circumstances were comfortable rather than affluent. The boyhood environment, as his friend Alfred Kroeber has well said, gave him "the rare and valuable attitude of being every man's inherent equal and no one's superior; a genuine ease and warmth, supplemented by his native kindness, in relations with people; an ability to subordinate himself to the majority, or circumstances, without withdrawing his independence of private judgment; and an even more outstanding faculty of directing others with their full loyalty and approval."

His early education was in private schools and by tutors, one of whom he was fond of citing as a master of pedagogy: Alsberg never forgot his first lesson in geography, taught not from a book but from the vantage point of Brooklyn Bridge. He entered Columbia University at the age of fifteen and proceeded after graduation to the College of Physicians and Surgeons, taking the degree of M.D. at the age of twenty-three. The practice of medicine, however, held lesser attractions than inquiry into things unknown. Yet the training in medicine stood him in good stead. Frequently in later years he referred with special satisfaction to the instruction he had received in psychiatry, whence sprang solution of personnel problems otherwise possibly baffling to administrators. Three years of his life after graduation from medical school were spent in Germany in study of the newly unfolding field of biochemistry. They were years made the more fruitful by his upbringing in a completely bilingual family where German was spoken as often as English.

From 1902 to 1908 Alsberg served successively as Assistant, Instructor, and Head in Physiological and later Biological Chemistry at Harvard Medical School. In 1908 he entered the government

service, first (until 1912) as Chemical Biologist in the Bureau of Plant Industry; next (until 1921) as Chief of the Bureau of Chemistry, United States Department of Agriculture. His marriage to Emma Blount Peebles, who survives him, occurred in 1912. He returned to academic life in 1921, as one of three Directors of the Food Research Institute at Stanford University. In 1938, as he approached the retirement age of sixty-five at Stanford, he resigned to become Director of the Giannini Foundation of Agricultural Economics at the University of California, where the university statutes allowed him five additional years of prospective activity.

Particularly in the closing decade of his life, he gave generously of his time and strength to participation and counsel beyond the routine of his academic appointments. He long served as Dean of Graduate Study at Stanford, and was a leading contributor to the activities of the Institute of Pacific Relations, the Social Science Research Council and its Pacific Coast Regional Committee, the Commission of Inquiry into National Policy in International Economic Relations, and the Board of Regents of Reed College.

Alsberg was a genuinely modest man. Speaking in 1938 to the graduating class at Reed College, he said (and meant): "I don't see anything in my career important enough to hold your attention for half an hour. . . . Life has treated me better than I deserve. . . . In me you see a Jack of many trades, a master of none. . . . Physician, biochemist, teacher, chemist, administrator, economist—I've had a fling at all of them in turn. It's been fun, but it hasn't made a great man of me. . . ."

It was fun in the sense that it made a career which sprang from and gave full scope for the two dominant aspects of his character—insatiable curiosity and indifference to financial success. Alsberg could have been a successful and wealthy physician, a great biochemist, a rich and famous consulting chemist. He chose not to cramp himself and not to specialize, but to blaze trails in unexplored country; not to become rich, but to live modestly. The choice was conscious and deliberate.

Although he chose to plow widely where he might have made deeper furrows, his plowing was by no means shallow. To all of his fields of inquiry he made genuine contributions. In the branches of natural science, his papers on the chemistry of proteins, on toxic substances, and on the physical and chemical properties of the starches have withstood the test of time. So also with his work in the social sciences, on the American baking industry, the fats and oils, population problems, relationships of weather to wheat yields, standards of living, and international relations.

Underlying Alsberg's breadth of interests and activities was his basic concern with a fundamental human problem, that of synthesizing cold fact with pure theory, progress in pure science and technology with progress in social science and human welfare. Possession of his peculiar combination of attitude and aptitude for this task is rare. Among the many students and associates of his varied career, it evoked affection and inspired respect and emulation in a degree accorded to few men.—M. K. BENNETT.

BIOCHEMICAL PROPERTIES OF "SALT-RISING" DOUGHS¹

MAX MILNER and W. F. GEDDES

Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota

(Read at the Annual Meeting, May 1942)

The leavening of bread doughs by the gas-producing function of nonpathogenic micro-organisms other than yeasts has received only minor commercial and scientific attention in recent years. In the pioneering days of the American West, however, yeast leavening was difficult to obtain and bacterial or so-called "salt-rising" fermentation was widely used in home bread production. The ferment was prepared by incubating a thin batter made with corn meal and fresh milk containing a little sugar and sodium bicarbonate for six to ten hours at 38°C. This starter was sponged with flour and incubated for an additional two to four hours, when the remaining ingredients, such as sugar, shortening, salt, and additional flour required to form the dough, were added and mixed. The dough was immediately placed in the pans and, after rising to approximately twice its original volume, it was baked in the usual manner. A baked loaf thus produced is of relatively small volume, with sharp corners and a smooth dark crust. The crumb is very close-grained and the bread has a unique aroma and taste somewhat resembling that of mild cheese.

Kohman (1912) studied salt-rising fermentation and showed that bacteria rather than yeasts were present in the fermenting sponges and doughs. He found that the bacterial flora predominating in a salt-rising starter depended on the temperature. At starter temperatures below 65°C a nonspore former, which did not liquefy gelatin, predominated and would successfully produce the bread. From the cultural characteristics given for this organism it would appear to be a strain of *Escherichia coli*. However, when corn meal starters were

¹ Paper No. 2004, Scientific Journal Series, Minnesota Agricultural Experiment Station, condensed from a thesis presented by Max Milner to the faculty of the Graduate School in partial fulfillment of the requirements for the M.S. degree, July, 1941.

prepared at temperatures of 75°–100°C, a spore-forming gelatin-liquefying organism predominated. As a result of these studies Kohman (U. S. Patent No. 1,149,839; 1915) developed a commercial preparation known as "Kohman's salt-rising yeast" for use in the production of salt-rising bread. In preparing this leaven, a fermenting flour sponge started at 75°–100°C is dried, ground, and diluted with corn meal, sodium bicarbonate, and calcium carbonate. The preparation is described as a pure culture of a rod-shaped facultative anaerobe which forms very resistant spores, liquefies gelatin, and grows well in milk and broth. From these and other cultural characteristics, it would appear to be a member of the aerobacillus group.

In studying the production of gas by the salt-rising leaven, Kohman found that casein was the most favorable substrate, followed by milk and then corn meal. The higher the content of protein degradation products in the casein, the sooner gas production appeared, but the addition of increments of lactose was essentially without influence. The gas resulting from the fermentation contained two volumes of hydrogen per unit volume of carbon dioxide. These observations indicate that carbohydrates are not involved in the gas-producing function of the ferment. Recent studies of dissimilation of carbohydrates by bacteria, reviewed by Harden (1932), Werkman (1939), Nord (1940), and Kalckar (1941), show that the ratio of hydrogen and carbon dioxide is never markedly in excess of unity.

The salt-rising bacteria bring about extensive proteolysis and the unique characteristics of the bread are doubtless attributable to this action. No intermediate fermentation is required after the dough ingredients are mixed with the fermenting sponge. Apparently the conditioning of the gluten, brought about by fermentation and punching in the instance of yeast doughs, is effected by the bacterial proteases. The publications of Sperry and Rettger (1915), Merrill and Clark (1928), Buchanan and Fulmer (1930), Hoogerheide and Weil (1939), Stephenson (1939), and others show that coli bacteria and aerobacillae, like other heterotrophic micro-organisms, including the yeasts, require adequate sources of available nitrogen for their metabolism. However they differ from the yeasts in that they secrete both endo- and exoproteases; the latter degrade the large protein aggregates of the medium to smaller fragments, which are assimilated and acted upon by the proteases and polypeptidases within the cells.

Bacterial proteases are inhibited by acids and work best in neutral or alkaline solutions; this has led many authors to classify them with the tryptases. Hoogerheide and Weil (1939) found that the exoproteases secreted by certain spore-forming anaerobes could be markedly activated by the addition of SH compounds in the presence of

traces of heavy metals. Such bacteria have been shown to produce a large number of fatty acids from casein as well as indole and skatole and a variety of amines of the putrefactive type. These products are undoubtedly responsible for the characteristic odor and flavor of salt-rising bread.

In the years since Kohman's investigations, cereal knowledge has been extended. The proteolytic nature of the salt-rising fermentation renders it of particular interest to study the effect of potassium bromate on the physical properties of the doughs, as measured by such instruments as the Brabender farinograph and extensograph, and to compare these properties with those of doughs made with yeast. The present study comprises an investigation of the comparative physical, physico-chemical, and chemical properties of salt-rising and yeast-leavened doughs. A method was developed for the experimental production of salt-rising bread and a study made of the effect of varying such factors as starter time, absorption, milk, sugar, potassium bromate, and pH. Comparisons of the physical properties, rates of gas production, reducing sugar, acidity, and amino nitrogen content of salt-rising and yeast-leavened doughs were made in order to elucidate certain differences between bacterial and yeast fermentation.

Experimental Materials and Methods

An 83% bleached patent flour of 10.8% protein and 0.40% ash content, on a 13.5% moisture basis, commercially milled from a blend of hard red spring and hard red winter wheats for the family trade, was used throughout the studies. Kohman's "salt-rising yeast," as supplied to the bakery trade, served as a source of the salt-rising ferment, while Fleischmann's yeast was employed for the yeast-leavened doughs. The milk solids were a standard spray-dried skim-milk powder manufactured for the baking trade.

An experimental technique for the production of salt-rising bread was developed and will be detailed later. In comparative tests of salt-rising and yeast doughs, the formulas were identical except for the leavening agent.

Measurements of gas production in salt-rising sponges and doughs were made in a slightly modified form of the standard pressuremeter apparatus described in *Cereal Laboratory Methods*. To reduce the error involved it seemed desirable to employ relatively large samples of starters and sponges. Accordingly somewhat larger pressuremeters than the regular type were constructed. As a measure of precaution, these were equipped with manometers capable of recording higher pressures, although in practice the pressures were released at frequent intervals. Upon calibration it was found that readings could be con-

verted to those of the standard type by use of the factor 1.28. However, as the primary interest was in relative rather than absolute gas production, the readings were not corrected. Because of the known temperature requirements of the bacteria, the tests were carried out at 38°C.

The reducing-sugar content of leavened and unleavened doughs was determined by a modification of the Blish-Sandstedt procedure for flour diastatic activity, as outlined in *Cereal Laboratory Methods*. By means of the Waring Blendor, 50 g of dough was dispersed in the required amount of combined sulfuric acid and buffer solution to give the solids-liquid ratio employed in the regular flour procedure. After two minutes the sodium tungstate solution was added and the Blendor operated for another minute. The suspension was centrifuged, the supernatant liquid filtered, and a suitable aliquot employed for the determination of reducing sugars by the regular ferricyanide procedure. The results were expressed as milligrams of maltose per dough weight equivalent to 10 g of flour containing 15% moisture.

In determining titratable acidity and amino nitrogen content, 300 g of the sponges or doughs were disintegrated with 500 ml of distilled water containing 2 g of sodium chloride in a Waring Blendor, operated for 2.5 minutes. A portion of the suspension was centrifuged and the titratable acidity and amino nitrogen content determined in 10 ml of the supernatant liquid by a modification of the Sorenson formal titration procedure suggested by Samuel (1934) using *N*/14 NaOH solution. This procedure, fully described in *Cereal Laboratory Methods* (4th ed., 1941), involves the use of phenol-red indicator and titration to pH 8.0, as determined by matching against a buffer solution of this pH in a comparator block.

Changes in the physical properties of yeast and salt-rising doughs during fermentation were followed by means of the Brabender farinograph and extensograph. The formulas and techniques employed will be detailed in a later section.

Experimental Baking Procedure for Salt-Rising Bread

In order to study conveniently the factors affecting salt-rising bread production it was necessary to develop a baking procedure employing 100 g of flour. The first trials, with a small-scale formula based on the method recommended for bread made with Kohman's prepared ferment, resulted in failure. Fermentation occurred in the starters and sponges but very little was apparent in the doughs. In studying numerous variations in formula and technique it was found that the addition of sodium bicarbonate at the sponge stage resulted in a marked increase in loaf volume. Various concentrations of other

buffer salts were then employed and the best results were secured with dibasic sodium phosphate to maintain a higher pH. The effects of varying dosages of this buffer salt on baking properties are shown in

TABLE I
EFFECT OF DIBASIC SODIUM PHOSPHATE ON LOAF CHARACTERISTICS

Dibasic sodium phosphate ¹	Loaf volume	External loaf characteristics	Internal loaf characteristics		
			Crumb grain	Crumb color	Crumb texture
0.0	385	Pale, bad shell top	Dense	Gray	Hard
0.3	420	Pale, shell top	Dense	Gray	Hard
0.6	420	Pale, shell top	Dense	Gray	Harsh
1.2	550	Good color, even break	Excellent	Good	Smooth
1.5	520	Good color and break	Good	Slightly open	Good

¹ Expressed as the percentage anhydrous salt based on the total flour and milk solids on a 15% moisture basis in the finished dough.

Table I. As a result of these preliminary studies, the following basic procedure for the experimental production of salt-rising bread was adopted.

Formula					
	Starter g		Sponge g		Dough g
Kohman's "yeast"	30	Flour	145	Flour	95
Milk solids (dry skim)	23	Dibasic sodium phosphate	8	Milk solids (dry skim)	3
Water	180 ml	(Na ₂ HPO ₄ ·12H ₂ O)		Sugar	5
		Starter	40	Shortening (hydrogenated)	5
		Water	118 ml	Salt	3
				Sponge	Entire amount

Fermentation Schedules

Ferment 9 hrs at 38°C	Ferment at 38°C until sponge begins to fall (1.75-3.0 hrs)	Place directly in tall form "100-g" experimental baking pans
-----------------------	--	--

Mix dough in the Hobart-Swanson mixer for 2 minutes. Proof 2.5 hrs at 38°C or until top of dough is level with top edges of the high sides of the pan. Bake for 25 minutes at 230°C ($\pm 5^\circ$).

The ferment and milk solids are vigorously stirred into the water, which has been brought to a boil in a 600-ml beaker. As the ferment hydrates slowly, stirring must be continued for several minutes to disperse any lumps that may form. The beaker is covered with a watch glass and placed in the fermentation cabinet.

In preparing the sponge, the sodium phosphate is dissolved in water at 50°C in a one-liter beaker. The flour and starter are added and mixed with a large flexible spatula. The beaker is covered with

a watch glass and the sponge fermented at 38°C until it has commenced to fall.

While the sponge is fermenting, the other dough ingredients are combined and warmed to a sufficiently high temperature (approximately 45°C) to give a final dough temperature of 38°C. When the sponge is ready to take, the warmed ingredients are transferred to the bowl of the Hobart-Swanson mixer, the sponge added, and the mixer operated for 2 minutes. The dough is removed from the mixer bowl and rounded up by folding 10 times in the hands. Two 200-g portions are quickly scaled off (to avoid excessive cooling) and immediately molded by the hand or rolling pin method as directed for the regular A.A.C.C. bread-baking test. The molded doughs are placed in lightly greased pans and proofed and baked as outlined in the above schedule. Loaf volume was measured one hour after removal from the oven and the loaves scored for external and internal characteristics the following day. Loaf type was judged by referring to the photo-standards of Blish (1928).

Notes: This formula differs somewhat from the recommended commercial procedure. A larger proportion of starter is used in the sponge and all the remaining water required for the dough is added at this stage; in addition, a buffer salt is employed in the sponge.

Because of the pronounced slackening of the doughs during proofing, they must be mixed at approximately 5% lower absorptions than yeast doughs.

The molding procedure does not appear to be as critical as with yeast doughs. The dough contains little or no gas and before gas production is under way during the proof, the dough settles to a smooth coherent mass in the bottom of the pans. The marked tendency of the doughs to flow during the proof period made the tall-form baking pans desirable. These pans provided greater support for the mobile doughs than the low-form pans and gave greater differentiation in crust character, break, and shape.

In studying the effect of absorption and potassium bromate, useful information was obtained by proofing one of the duplicate doughs to height and the other to time. Thus an increase in absorption shortened, and potassium bromate lengthened, the time required to proof to height.

The sensitivity of the doughs to under- or over-fermentation was manifested principally in the loaf type, which ranged from pale shell-topped loaves to loaves with dark crusts and no break.

The procedure was found to give consistent and readily reproducible results both within and between days. In the majority of cases the volumes of duplicate loaves checked within 5 cc.

Effect of Variations in Formula and Procedure on Baking Properties

With the basic formula and procedure outlined above, a study was made of the influence of variations in formula and procedure on baking properties.

Fermentation time of starter: The starter fermentation time was varied from 6 to 11 hours in one-hour increments. The results showed that satisfactory loaves could be baked with starters fermented from 6 to 11 hours, with an optimum time of 8 to 9 hours.

Absorption: Absorption studies, summarized in Table II, showed that a shorter proofing time was required to secure optimum bread as the absorption was increased. When the optimum proof time was employed for each absorption, there was little difference in loaf volume and other bread characteristics. However, the doughs made at the highest absorptions were very mobile, sticky, and difficult to handle.

TABLE II
EFFECT OF ABSORPTION ON PROOF RATE AND LOAF CHARACTERISTICS¹

Absorption, %	Time of proof		Oven spring	Volume	Loaf type	Crust color	Crumb		
	To time	To weight					Grain	Texture	Color
	10:00	10:10	cm	cc					
52	150	—	1.5	535	J9	S	9	8	9
	—	175	1.5	550	J9	S	9	8	9
54	150	—	1.5	560	J10	S	9	8	9
	—	155	1.8	570	J10	S	9	8	9
56	150	—	2.0	530	J10	S	9	8	9
	—	180	1.3	535	J10	S	9	8	9
58	150	—	0.8	555	J7	S	6	9	9
	—	110	1.8	555	J10	S	6	9	9
60	150	—	0.5	555	J6	S	6	9	9
	—	125	1.5	555	J9	S	8	9	9

¹ Crumb grain, texture, and color judged on the basis of 10 as a perfect score. S = satisfactory.

² Absorption is expressed on the basis of the combined weight of flour and milk solids (15% moisture basis) in the dough.

Effect of milk: Kohman (1912) found that starters cultured without milk developed more slowly but gassed for a longer period; the increase in acidity of such cultures was less than when milk was included. Baking tests were accordingly conducted in which increments of skim-milk powder were added to the starter only, and to sponges and doughs, prepared with starter, containing the minimum quantity of milk that would provide for its adequate development in about 12 hours. Typical results are shown in Table III.

Starters cultured without milk were very slow in developing and required approximately 16 hours of fermentation before they were ready for making up the sponges. Sponges prepared from milk-free starters were also slow, taking from 5 to 6 hours to reach optimum development. However, the doughs made entirely without milk proofed as rapidly as did those containing it. Significantly, the milk-free loaves were completely lacking in the cheeselike odor and taste of salt-rising bread.

Milk added at one stage either to starters or to sponges and doughs made up from minimal milk starters gave loaves inferior to those obtained by the basic formula in which milk was added to both the starter and the dough. Great tolerance to wide variations of milk

TABLE III
EFFECT OF MILK SOLIDS ON SPONGE TIME, PROOF RATE,
AND LOAF CHARACTERISTICS

Milk added				Proof time			Oven spring	Loaf vol- ume	Loaf type	Crust color ²	Crumb		
Starter	Sponge	Dough	Total	Sponge time	To time	To height					Grain	Tex- ture	Color
				min	min	min	in	cc	(10)		(10)	(10)	(10)
0.0	—	0.0	0.0	330	—	140	1.0	535	J10	P	9	10	10
BASIC FORMULA													
1.0	—	1.2	2.8	150	150	—	1.3	550	J10	S	9	9	8
MILK ADDED TO STARTER ONLY													
0.48	—	—	0.48	240	120	—	3.0	535	I8	P	10	9	10
0.48	—	—	0.48	240	—	180	1.0	545	J9	P	8	7	9
1.44	—	—	1.44	170	120	—	3.6	495	H7	SI P	10	9	10
1.44	—	—	1.44	170	—	315	1.0	555	J8	S	7	7	7
2.40	—	—	2.40	180	120	—	3.6	480	H7	SI D	10	9	10
2.40	—	—	2.40	180	—	305	1.0	545	J8	SI D	8	7	8
3.35	—	—	3.35	150	120	—	3.8	490	H7	D	10	9	10
3.35	—	—	3.35	150	—	230	1.3	545	J8	D	8	7	8
4.80	—	—	4.80	125	120	—	3.8	515	H8	D	10	9	10
4.80	—	—	4.80	125	—	235	1.3	550	J9	VD	8	7	8
MILK ADDED TO STARTER AND SPONGE													
0.48	1.15	—	1.63	190	150	—	4.3	460	H6	SI P	9	8	10
0.48	1.15	—	1.63	190	—	250	1.8	480	J8	D	8	8	8
0.48	2.32	—	2.80	165	150	—	4.3	515	H8	S	9	8	10
0.48	2.32	—	2.80	165	—	450	0.5	490	J6	D	8	7	7
0.48	3.42	—	3.90	165	—	—	Dough failed to ferment						
MILK ADDED TO STARTER AND DOUGH													
0.48	—	1.15	1.63	140	150	—	4.6	460	H6	SI P	9	9	10
0.48	—	1.15	1.63	140	—	195	1.3	525	J9	S	10	9	9
0.48	—	2.32	2.80	195	150	—	4.6	465	H6	S	9	9	10
0.48	—	2.32	2.80	195	—	255	2.0	480	J6	D	7	7	7
0.48	—	3.42	3.90	255	150	—	2.3	505	J9	D	9	10	9
0.48	—	3.42	3.90	255	—	410	1.0	505	J7	D	7	7	7

¹ The milk percentages given for the starter and sponge are expressed on the total flour and milk added at 15% moisture basis in the finished dough.
² D = dark; P = pale; S = satisfactory; SI = slightly; V = very.

content was shown when milk was added at the starter stage. Thus doughs with 0.048% and 4.8% milk solids (total flour and milk basis) added to the starter showed little difference in proofing time or loaf characteristics except for a darkening of the crust color with increasing milk content. However, when milk was added at the later stages of fermentation, particularly to the dough, progressive inhibition of fermentation was manifested with increasing milk content.

Effect of sucrose: The results of baking tests in which sucrose was added to sponges and doughs in quantities varying from 0% to 6% (total flour and milk solids basis) in 2% increments are shown in Table IV. Sucrose had little effect on dough proof time or loaf prop-

TABLE IV
EFFECT OF SUCROSE ON PROOF RATE AND LOAF CHARACTERISTICS

Sucrose dosage	Proof time		Open spring	Loaf volume	Loaf type	Crust ² color	Crumb		
	To time	To height					Grain	Texture	Color
%	min.	min.	cm.	cc					
0	150	—	0.3	550	J $\frac{1}{2}$	P	4	7	8
	—	95	2.0	545	J9	VP	10	9	9
SUCROSE ADDED TO SPONGE									
2	150	—	1.0	530	J7	P	6	8	8
	—	105	1.3	545	J9	P	10	9	9
4	150	—	0.0	530	J5	S-P	5	7	8
	—	95	1.5	575	J8	S-P	9	9	9
6	150	—	1.0	525	J7	S	7	8	8
	—	100	1.5	550	J8	S	9	9	9
SUCROSE ADDED TO DOUGH									
2	150	—	1.5	535	J10	S	10	9	9
	—	150	1.5	535	J10	S	10	9	9
4	150	—	1.5	440	H7	D	8	8	10
	—	240 ³	3.3	480	J8	D	8	8	9
6	150	—	4.8	480	H7	VD	8	9	10
	—	240 ³	2.5	490	J6	VD	9	8	9

¹ Sucrose percentages given are based on the combined weights of flour and milk solids (both containing 15% moisture) in the dough.

² See footnote 2, Table III.

³ These doughs did not proof to height by this time.

erties when used in the sponge but appeared to retard the fermentation when added at the dough stage. Thus the doughs to which 6% sucrose was added could not be proofed to height even after 4 hours, and those baked after only 2.5 hours of proof gave typically under-fermented loaves.

Effect of potassium bromate: The results of experimental baking tests, in which nil, 0.001%, 0.003%, 0.007%, and 0.009% potassium bromate (calculated on the basis of the total flour and milk solids in the dough) were superimposed on the basic formula at the sponge stage, are recorded in Table V. This reagent markedly checked the characteristic tendency of salt-rising doughs to slacken with fermentation. The improvement in stability with increasing increments of bromate was accompanied by progressive decreases in loaf volume

when the doughs were proofed for a fixed time; moreover, the times required to proof to height were greatly increased. These observations strongly indicate that the bromate depressed both the proteolytic and gassing functions of the bacteria.

TABLE V
EFFECT OF POTASSIUM BROMATE ON PROOF RATE AND LOAF CHARACTERISTICS

KBrO ₃	Proof time		Oven spring	Loaf volume	Loaf type	Crust color	Crumb		
	To time	To height					Grain	Texture	Color
0.0	150	—	1.5	600	J8	S	8	8	9
	—	110	1.5	350	J10	S	9	9	9
1.0	150	—	3.3	575	G9	S	10	10	10
	—	140	2.5	600	G9	S	9	9	9
3.0	150	—	3.3	480	H8	S	9	9	10
	—	345 ^a	3.3	610	G10	S	8	9	8
7.0	150	—	3.0	390	H5	VP	7	8	8
	—	330 ^b	3.6	495	G7	P	7	9	7
9.0	150	—	3.3	375	H5	VP	6	7	8
	—	340 ^b	3.8	405	H7	P	7	7	7

^a Loafage of potassium bromate is given as mg per 100 g flour and milk solids (each containing 15% moisture).

^b See footnote to Table III.

^c Doughs did not proof to height in this time.

The inclusion of bromate in the sponge was reflected in increased oven spring and change in loaf symmetry from the J type with a small break to the G type with a bold top and large break.

Biochemical Properties

The results of the baking tests suggest that the role of sucrose in the salt-rising fermentation is quite different from that in yeast metabolism. Moreover, the inclusion of bromate in the formula not only resulted in a decrease in proteolysis, as indicated by a lowered tendency of the doughs to slacken with fermentation, but also decreased the rate of gas production, as reflected in the longer times required for the bromated doughs to proof to height. These observations made it of interest to conduct comparative tests of factors influencing pH, the rate of gas production, changes in sugar level, and the extent of proteolysis in salt-rising and yeast doughs.

Effect of dibasic sodium phosphate on pH: Changes in pH during the fermentation of starters, sponges, and doughs, prepared according to the basic method, were followed by means of a glass electrode. Parallel tests were conducted on a sponge made without the addition of phosphate and also on a dough prepared therefrom (Table VI).

In the starter the acidity increased markedly between the third and sixth hours, indicating rapid bacterial growth during this period. The buffering effect of dibasic sodium phosphate in the sponge is clearly shown and the higher pH's resulting from its use are evident

TABLE VI
EFFECT OF FERMENTATION ON pH OF STARTER, SPONGES, AND DOUGHS

Fermentation time hrs	Starter	Sponge		Dough ¹	
		Without phosphate buffer	With phosphate buffer	Without phosphate buffer	With phosphate buffer
0	—	6.3	7.5	5.6	6.3
1	7.8	5.8	7.2	5.5	6.1
2	7.6	5.4	6.8	5.4	6.0
3	7.3	5.1	6.3	5.3	5.8
4	6.9	5.0	5.8	5.3	5.8
5	6.5	5.0	5.6	—	—
6	6.1	5.0	5.6	—	—
7	5.8	—	—	—	—
8	5.7	—	—	—	—
9	5.5	—	—	—	—
10	5.4	—	—	—	—
11	5.4	—	—	—	—
24	5.1	4.8	5.1	4.9	5.2

¹ Doughs were prepared from sponges made with and without phosphate buffer which were fermented for 2½ hours

in the dough. It is notable that the increased pH in the dough resulting from the presence of phosphate is only in the order of 0.5 at the third hour—that is, at the completion of the normal proofing period. The higher pH, however, is reflected in marked improvement in bread quality.

Gas-production studies: Gas-production tests were carried out in duplicate at 38°C by the pressuremeter method, with 78 g of starter prepared in the regular manner outlined in the baking test procedure. The mean cumulative manometric readings at hourly intervals for the period 0–12 hours are shown in Figure 1. The quarter-hourly and half-hourly rates calculated from the data are plotted in Figure 2. In view of the large volume of gas produced, it became necessary to release the pressure several times. It will be noted that even relatively low pressures markedly decreased the rate of gas production, as indicated particularly by the increased rates following release of the gas. Similar effects of pressure were noted in gas-production studies with yeast sponges when the pressure exceeded about 100 mm of mercury. These results are not in accord with the observations of Sandstedt and Blish (1936) on yeast doughs, who found that the rate

of fermentation was not significantly influenced by changes in pressure over a wide range.

Salt-rising sponges were prepared with and without dibasic sodium phosphate in 9-hour starters, according to the formula given under the baking test procedure. Thirty-gram portions of the sponges were

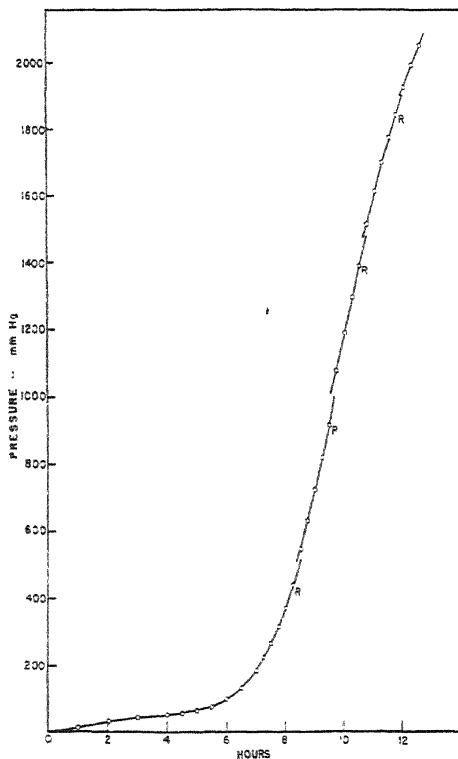


Fig. 1. Total gas production by salt-rising starter after varying fermentation times showing the effect of pressure.

made up in duplicate in 100-ml beakers, which were then placed in the pressuremeters. Zero time was taken 10 minutes after immersion of the pressuremeters in the constant-temperature bath (38°C). The pronounced stimulating effect of phosphate on the fermentation is shown by the mean gas-production curves in Figure 3. At the end of eight hours, the phosphated sponge gave a pressure of 623 mm of mercury, as contrasted with 341 mm for the nonphosphated sponge. Rate curves computed from the data showed that the addition of

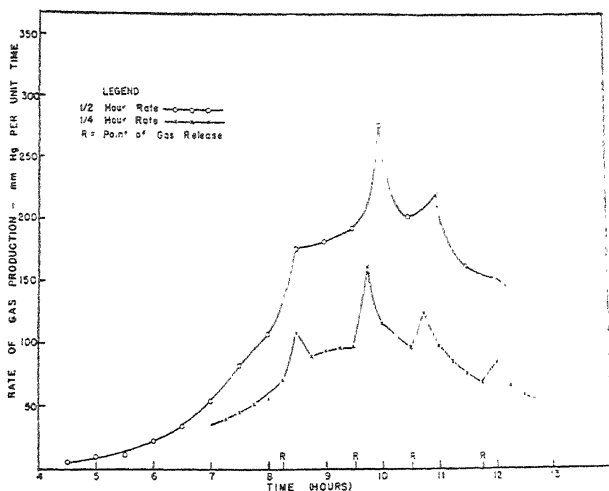


Fig. 2. Rates of gas production by salt-rising starter at various fermentation times showing the effect of releasing gas pressure.

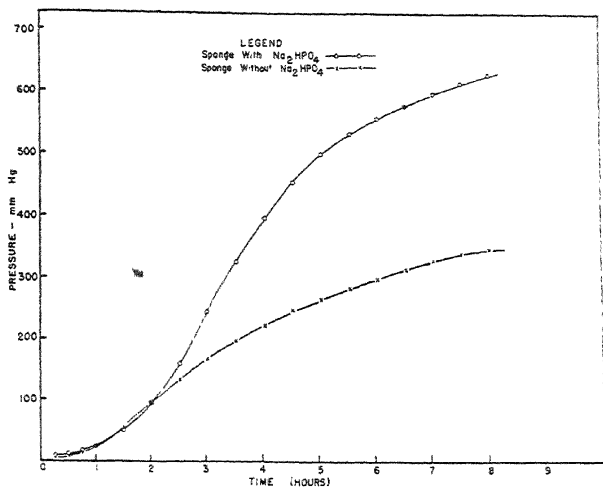


Fig. 3. Effect of dibasic sodium phosphate on gas production in salt-rising doughs.

phosphate shifted the maximum rate of gas production from the second to the third hour of fermentation.

The effect of potassium bromate on gas production was determined with sponges containing nil, 0.007%, and 0.014% potassium bromate, respectively. These bromate levels are expressed on the basis of the

flour and milk solids present in the sponge and are equivalent to 0.004% and 0.008% when expressed on the total flour and milk solids in the dough. The mean gas production curves for duplicate determinations on 30-gram sponges are shown in Figure 4. These show

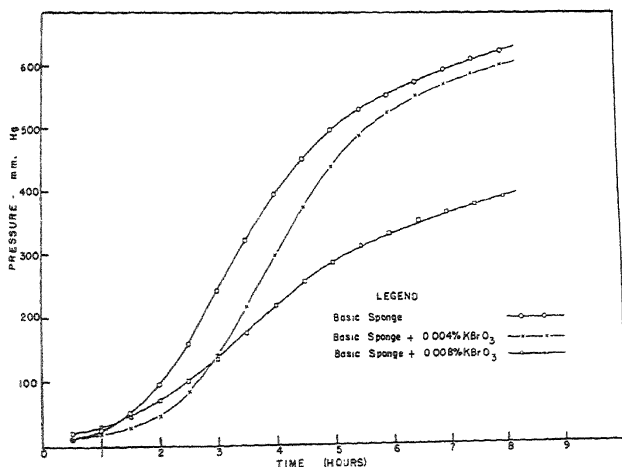


Fig. 4. Effect of potassium bromate on gas production in salt-rising sponges. The bromate concentrations shown are based on the total flour and milk solids used in making the dough.

that the lower bromate dosage had a slight retarding effect on gas production, while the higher dosage had a pronounced inhibitory effect. A study of the gas production rates revealed that the bromated sponges exhibited maximum rates at about 4 hours of fermentation as compared with 3 hours for the nonbromated sponge.

TABLE VII
EFFECT OF DIBASIC SODIUM PHOSPHATE ON GAS PRODUCTION IN
SALT-RIISING DOUGHS

Fermentation time	Mercury pressure	
	Without phosphate	With phosphate
hr:	mm	mm
0.5	30	32
1.0	45	56
1.5	59	81
2.0	68	106
2.5	76	129
3.0	82	151
3.5	86	170
4.0	90	187
4.5	94	200
5.0	97	213
5.5	101	224
6.0	103	235

Gas-production tests were made with 40-g portions of salt-rising doughs prepared as outlined in the baking procedure and also with the omission of the phosphate. The mean results of duplicate determinations (Table VII) show a marked increase due to phosphate and serve to explain its beneficial effect on baking properties.

In order to compare rates of gas production in salt-rising and yeast doughs, a 3% yeast dough (containing the same proportions of flour, salt, sugar, and milk solids) was prepared by a sponge and dough method analogous to that employed in making the basic salt-rising dough. Forty-gram portions were fermented in the pressuremeters at 30°C. The mean results of duplicate determinations of gas production, recorded in Table VIII, together with data for the same

TABLE VIII
RELATIVE GAS PRODUCTION IN SALT-RISING AND YEAST DOUGHS¹

Time	Mercury pressure	
	Basic salt-rising dough	3% yeast dough
<i>hrs</i>	<i>mm</i>	<i>mm</i>
0.5	32	121
1.0	56	252
1.5	81	371
2.0	106	482
2.5	129	591
3.0	151	668
4.0	187	778
5.0	213	825

¹ Salt-rising dough fermented at 38°C, yeast dough at 30°C.

quantity of salt-rising dough fermented at 38°C, show that the yeast doughs produced more gas than salt-rising doughs of similar composition.

Reducing sugar levels in salt-rising and yeast doughs: Kohman (1912) observed that the addition of lactose to a casein culture of salt-rising bacteria did not stimulate gas production. In the present studies sugarless salt-rising sponges and doughs fermented and proofed almost as rapidly as those containing added sucrose. These findings indicate that sugar is not involved in the production of gas by the bacteria. Accordingly a comparative study was made of the reducing-sugar levels in salt-rising and yeast doughs made without added sucrose. The following doughs were prepared at 55% absorption:

1. Sugarless salt-rising dough. The basic formula was used except that sugar was omitted.
2. No-starter, sugarless, salt-rising dough. The dough contained the ingredients of the preceding dough but was made up from a sponge to which no starter or dibasic sodium phosphate had been added.

3. Sugarless straight yeast dough. A 3% straight yeast dough made without sucrose, but containing the same proportions of salt, milk solids, and shortening as the salt-rising doughs.

4. Sugarless yeast dough, sponge method. This dough was made by the same formula as the straight yeast dough but 60% of the flour, 50% of the milk solids, and all the water and yeast were sponged and fermented at 30°C for three hours prior to making up the dough.

The salt-rising doughs were kept at 38°C and the yeast doughs at 30°C; reducing-sugar determinations were made at intervals of 0, 1, 2, 3, and 4 hours by the method previously outlined. The results, presented graphically in Figure 5, indicate little or no sugar consump-

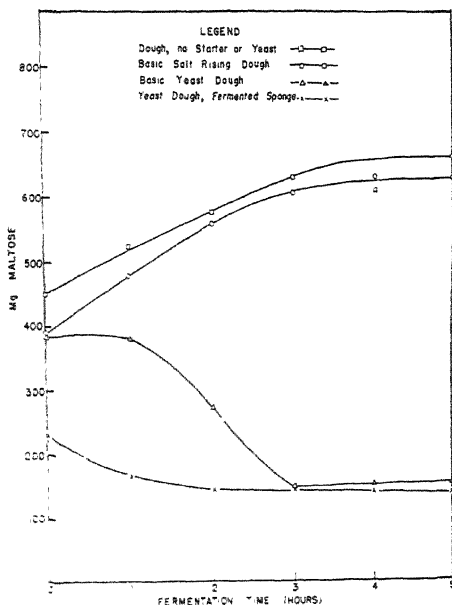


Fig. 5. Changes in reducing sugar content of salt-rising and yeast doughs with fermentation. The maltose content is expressed as milligrams of maltose per dough weight equivalent to 100 g flour and milk solids (15% moisture basis).

tion by the bacteria. The reducing sugar contents of the nonfermenting and fermenting salt-rising doughs were nearly equal and increased with time as a result of amylase activity. In striking contrast, yeast fermentation rapidly lowered the reducing-sugar content of the yeast doughs to a low level.

Titrateable acidity and proteolytic activity: The formol titration procedure furnished an index of both the titrateable acidity and amino nitrogen content of sponges and doughs at various stages of fermentation.

Acidity determinations on salt-rising sponges prepared in the regular manner, with the omission of phosphate, starter, and milk solids, and with the addition of 0.004% potassium bromate (expressed on the basis of total flour and milk solids in dough), are shown graphically in Figure 6. In the control sponge, made without starter but con-

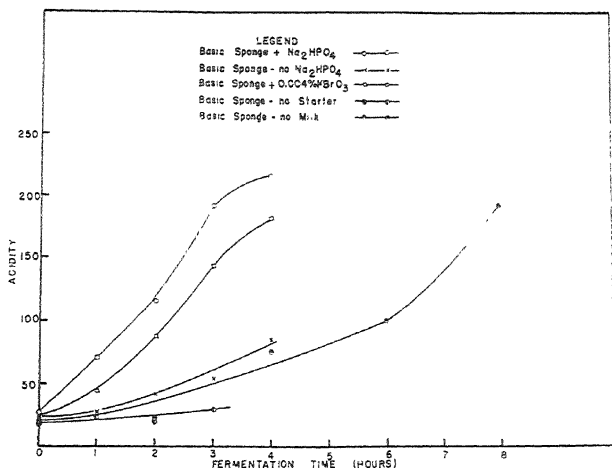


Fig. 6. The development of titratable acidity in salt-rising sponges showing the effect of sodium phosphate, potassium bromate, and milk solids. Titratable acidity is expressed as ml N/14 NaOH/100 g flour and milk solids in the sponge.

taining all other ingredients including phosphate, the titratable acidity increased only very slightly with time. The highest development of acidity was given by the basic sponge and the omission of phosphate resulted in a very marked decrease. Bromate slightly depressed the acidity. The curve for the sponge made from starter in which milk was omitted is in line with the very long period required for the development of sponges noted in the baking studies.

Titratable acidity was followed in a basic salt-rising dough prepared in the regular manner, in a basic dough plus 0.004% potassium bromate (total flour and milk solids basis, bromate added to sponge), and in a dough made without the use of starter in the sponge. A comparison of the results given in Table IX with those obtained for sponges (Fig. 6) shows that the differences in titratable acidity of sponges due to bromate are considerably reduced in the dough.

The titratable acidity values presented above represent the first stage of the formol titration, and hence amino nitrogen values were obtained for the same sponges and doughs. The mean results for the

TABLE IX
TITRABLE ACIDITY OF SALT-RISE DOUGHS

Fermentation time	N 14 NaOH per 100 g flour and milk solids		
	Basic dough less starter	Basic dough	Basic dough +0.004% KBrO ₃
<i>ml</i>	<i>ml</i>	<i>ml</i>	<i>ml</i>
0	19.5	115.5	110.0
1	22.4	131.0	123.0
2	26.1	146.0	134.5
3	27.8	162.0	149.5
4	31.4	175.0	163.0

AMINO NITROGEN PER 100 G FLOUR AND MILK SOLIDS			
<i>hrs</i>	<i>mg</i>	<i>mg</i>	<i>mg</i>
0	30.7	51.6	49.2
1	31.7	62.3	59.4
2	32.2	73.6	68.7
3	32.4	80.4	76.0
4	28.8	86.0	82.6
5	30.0	93.5	87.5

sponges, shown graphically in Figure 7, reveal that proteolysis is most extensive in the basic sponge and is markedly inhibited by bromate. In the absence of phosphate, proteolysis proceeded initially at a more

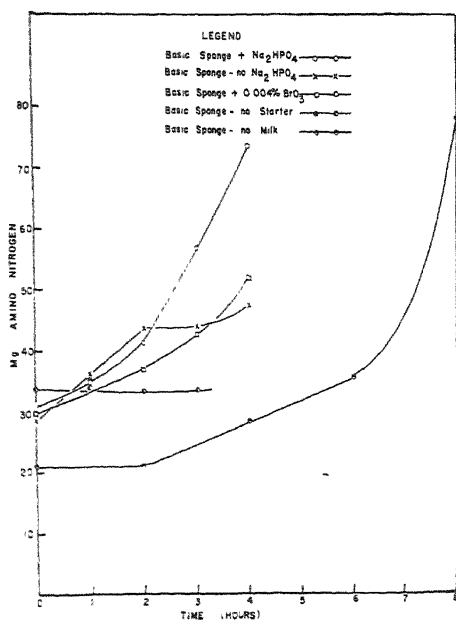


Fig. 7. Proteolysis in salt-rising sponges showing the effect of sodium phosphate, potassium bromate, and milk solids. Amino nitrogen content is expressed as mg per 100 g flour and milk solids in the sponge.

rapid rate than in the sponge containing the buffer salt but fell off sharply after two hours. This decrease in rate is doubtless associated with the general loss in fermentative activity noted in the previous tests. Similarly the low proteolytic activity in the milk-free sponges is correlated with the low rate of increase in titratable acidity and the slow sponge development observed in the baking tests when no milk was used.

The data in Table IX reveal a slight increase in amino nitrogen content of doughs made from sponges containing no starter (due probably to the inherent flour proteases) up to the third hour; the subsequent slight drop may be attributed to the activity of wild yeasts. With regard to the influence of bromate, the initial level of amino nitrogen was lower in the bromated dough as a result of the inhibition of proteolysis during the sponge stage where the bromate was added. In the bromated and nonbromated doughs, however, the rates of amino nitrogen production were virtually identical. A comparison of these data with those obtained by Shen and Geddes (1942) for yeast doughs in which utilization of the amino nitrogen by the yeast was prevented by the addition of octyl alcohol, shows that proteolysis in salt-rising doughs is much greater.

Effects of Salt-Rising and Yeast Fermentation on Physical Properties of Doughs

As a measure of the relative effects of salt-rising and yeast fermentation on the physical properties of doughs, a series of tests was made with the Brabender farinograph and extensograph.

For the farinograph tests, a basic salt-rising dough, a similar dough containing 0.004% potassium bromate (added at the sponge stage), and a nonbromated straight-yeast dough made by a formula containing 3% yeast and the same percentages of sugar, salt, shortening, and milk as the salt-rising doughs, were used. All three doughs were mixed to 55% absorption, divided into 80-g aliquots, rounded up and fermented in covered 300-ml beakers for varying times. The salt-rising doughs were fermented at 38°C, the yeast doughs at 30°C and farinograms were made in the small mixer at 30°C. The consistencies recorded after 10 minutes of mixing are given in Table X. While the Brabender consistency values of all three doughs markedly decreased with fermentation, the relative values were in striking contrast to the observed consistency as reflected in handling properties. The zero time consistency of the yeast dough was 20 units less than for the salt-rising doughs, and the consistency decreased at a more rapid rate than did that of both salt-rising doughs. Yet, at zero time, 60% of the flour used in the salt-rising doughs had already undergone 150

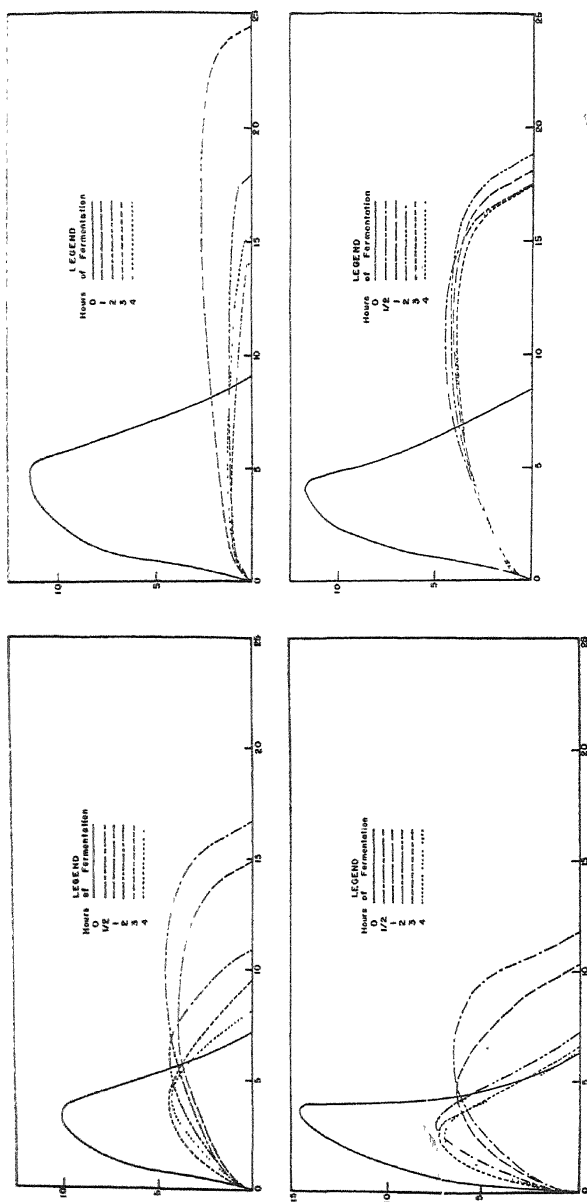


Fig. 8. Extensograms for bromated and nonbromated salt-rising and yeast doughs after different fermentation times. Extensograms for yeast doughs are shown in the left-hand column and for salt-rising doughs in the right-hand column. Those in the top row are for nonbromated doughs and those in the bottom row are for bromated doughs. The graphs were made from direct tracings of the extensograph curves.

minutes of fermentation in the sponge and these doughs were noticeably of somewhat lower consistency than the yeast dough. At the 5-hour fermentation time the nonbromated salt-rising dough could be poured from the beaker as a result of extensive proteolysis, but it exhibited a higher Brabender consistency by 80 units than the yeast

TABLE X
EFFECT OF SALT-RISING AND YEAST FERMENTATION ON BRABENDER
CONSISTENCY VALUES

Fermentation time <i>hr.</i>	Salt-rising dough		Yeast dough
	Without bromate	With 0.004% bromate	
	<i>Brabender consistency units</i>		
0	500	500	480
1	470	480	440
2	450	460	420
3	440	430	380
4	430	420	360
5	420	420	340

dough fermented for the same time. Moreover, the bromated salt-rising doughs were observed to be much stiffer than the nonbromated doughs, particularly as the fermentation time was extended. It would appear that the farinograph fails to interpret the great decrease in consistency of the salt-rising doughs with fermentation because of their increased stickiness.

Extensograms were made with salt-rising and yeast doughs prepared with and without 0.004% potassium bromate. The formulas were the same as those employed for the farinograph tests with the exception that only 2% yeast was used in the yeast doughs; this decrease was found necessary in order to secure better replication in the extensograph. In the instance of salt-rising doughs, six 150-gram aliquots taken from a large mix were scaled off, rounded up, molded with the auxiliary extensograph equipment, and placed in the dough holders. An extensogram was made with one aliquot immediately after mixing while the others were reserved for tests after fermenting at 38°C for various times. In the instance of the yeast doughs, the aliquots were fermented in covered beakers at 30°C until 30 minutes before the end of the fermentation period. At this time the doughs were rounded, molded, and clamped in the dough holders. Extensograms were made after fermenting in the holders for 30 minutes at 30°C. Photographs of direct tracings of the extensograms are reproduced in Figure 8. Munz and Brabender (1940) have shown that differences in the physical properties of doughs as a result of various treatments are best reflected by the extensograph when studied after a period of rest. When tested immediately after mixing, the "excited

state" of the doughs greatly influences the character of the extensogram. This observation is fully confirmed here. The extensograms graphically illustrate the marked change in extensibility of the salt-rising doughs with fermentation and the increase in stability resulting from the inclusion of potassium bromate in the formula. A notable feature of these curves is the narrow range of the vertical component (stress) and the great variability in the horizontal component (strain) with increasing fermentation time. Both the nonbromated and bro-

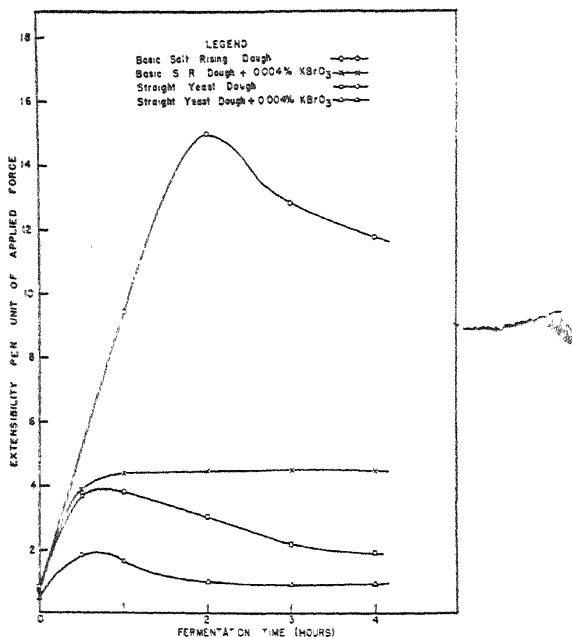


Fig. 9. Changes in extensibility E/F ratios for bromated and nonbromated salt-rising and yeast doughs with fermentation.

mated yeast doughs exhibit a much lower extensibility than the salt-rising doughs at the first hour of fermentation and a gradual "tightening" or decrease in extensibility upon further fermentation. The "tightening" effect of bromate, noted for the salt-rising doughs, is also evident with the yeast doughs. The relative changes in the physical properties of the salt-rising and yeast doughs with fermentation and the effect of bromate may be clearly shown by computing the strain values per unit stress or, using the terminology of Munz and Brabender, the "extensibility (E) per unit of maximum force (F). The E/F ratios are shown in Figure 9.

Discussion

The marked differences in physical and biochemical properties between salt-rising and yeast doughs can be ascribed to the distinctive metabolism of the fermentative organisms involved. Salt-rising fermentation is marked by a high degree of proteolysis which results in doughs and loaves possessing unique properties. The structure and properties of such doughs and loaves are highly sensitive to overproof, the doughs exhibiting a high rate of production and increasing content of amino nitrogen with fermentation.

Buchanan and Fulmer (1930) make this statement: "The fact that bacterial proteases are inhibited by acids and work best in neutral or alkaline solutions has led to their being classified with the tryptases by many authors." If it is assumed that the proteolytic action of salt-rising fermentation can be ascribed to the secretion of such tryptic type enzymes by the bacteria involved, the beneficial effects obtained by the inclusion of the basic buffering salt on the fermentative activity may be accounted for. The higher pH levels (5.5-7.5) required for optimum fermentative activity appeared necessary not only for the proteolytic function but for the gas-producing function as well. The organisms were found to utilize little if any reducing sugars in their gas-producing function, which is apparently connected with protein decomposition and utilization.

Proteolytic micro-organisms produce a large variety of organic acids as well as intermediate protein decomposition products. Anaerobic spore formers, into which class salt-rising organisms probably fall, are known to produce a variety of amines by their action on casein. The characteristic odor and taste of salt-rising bread, when it includes milk solids in its makeup, may accordingly be accounted for. In these studies, salt-rising loaves prepared without milk solids completely lacked the characteristic odor.

Potassium bromate in relatively high concentrations markedly inhibited proteolysis in the sponge. As gas production and titratable acidity were also lower, the bromate appeared to retard the metabolic activity of the organism as a whole. At the dough stage, in which the bromate concentrations were lower than in the sponge, the rate of increase in amino nitrogen was virtually identical for the bromated and nonbromated doughs. The dough-handling properties and stability to fermentation were markedly superior when bromate was added at the sponge stage. The regular doughs became slack and runny after 2 hours' fermentation, yielding flat-topped loaves with coarse open grain. Similar doughs containing 0.001% to 0.003% potassium bromate (total flour and milk solids basis) added to the sponge re-

mained stable up to $5\frac{3}{4}$ hours of fermentation and yielded loaves of large volume for this type of bread. These large volumes were obtained in spite of the lower gas production and indicate increased gas retention in the bromated doughs.

It is of interest to note that the farinograph failed to record the pronounced slackening of the salt-rising doughs or the "tightening effect" of potassium bromate. In fact the farinograph indicated that salt-rising doughs, after several hours of fermentation, when they would actually flow from the vessels in which they were contained, possessed a greater consistency than yeast doughs which were much firmer and of higher stability at similar fermentation periods. This anomaly is probably due to the increasing stickiness of the salt-rising doughs with fermentation. In striking contrast, the extensograph recorded differences in the physical properties of the salt-rising and yeast doughs which were in line with their observed physical condition. Furthermore this instrument clearly interpreted the marked stabilizing effect of bromate on dough structure and also showed that the effect of this reagent was more marked in salt-rising than in yeast doughs.

Summary

Employing a commercial preparation, known as Kohman's "salt-rising yeast," which is prepared from milk cultures of certain spore-forming bacteria present in corn meal, a study has been made of the optimum conditions for baking and the biochemical properties of the doughs.

A successful laboratory-scale procedure for the production of salt-rising bread required certain modifications of recommended commercial practice for the preparation of the starter, sponge, and dough. Satisfactory loaves were obtained by using twice the quantity of ferment recommended, by adding all the water called for by the dough formula to the sponge, and maintaining the pH at levels above 5.8 by the use of dibasic sodium phosphate as a buffering agent.

Salt-rising bread is characterized by a relatively small loaf with square corners and a smooth dark crust. The crumb is compact, of even, fine grain and smooth texture, with a characteristic cheese-like odor.

Salt-rising doughs markedly decrease in consistency with fermentation and are sensitive to overproof. Increased absorption and use of milk in the starter and sponge shortened the proof time required for optimum bread. Sucrose was without effect while potassium bromate lengthened the proof time. Starters and sponges prepared without milk fermented slowly and milk-free bread lacked the characteristic cheese-like odor and flavor of salt-rising bread.

Biochemical studies showed that little or no sugar is utilized in the production of gas by the bacteria. The fermentation is characterized by a high rate of proteolysis resulting in a marked slackening of the doughs during fermentation. Potassium bromate added at the sponge stage markedly decreased the rate of proteolysis as well as the rate of gas production and the development of acidity. Doughs prepared from bromated sponges exhibited less slackening than nonbromated doughs with fermentation.

The farinograph failed to record the marked slackening of salt-rising doughs with fermentation and the stabilizing effect of potassium bromate. On the other hand the extensograph clearly revealed the effects of fermentation and bromate as well as the markedly greater extensibility of salt-rising as compared with yeast doughs.

Acknowledgment

The authors are indebted to H. A. Kohman, Mellon Institute, Pittsburgh, for his kindness in supplying the salt-rising ferment used in these studies and a recommended formula for the commercial production of salt-rising bread.

Literature Cited

- Blish, M. J., *et al.*
1928 Report of committee on standardization of the experimental baking test. *Cereal Chem.* 5: 277-300.
- Buchanan, R. E., and Fulmer, E. I.
1930 Physiology and biochemistry of bacteria. The Williams and Wilkins Co., Baltimore.
- Harden, A.
1932 Alcoholic fermentation. Longmans, Green and Co., London.
- Hoogerheide, J. C., and Weil, L.
1939 Studies on the metabolism and proteolytic enzymes of the strict anaerobes. *J. Franklin Inst.* 228: 519-520.
- Kalckar, H. M.
1941 The nature of energetic coupling in biological synthesis. *Chem. Reviews* 28(1): 71-178.
- Kohman, H. A.
1912 Salt-rising bread and some comparisons with bread made with yeast. *Ind. Eng. Chem.* 4: 20-30 and 100-106.
- Merrill, Alice T., and Clark, W. M.
1928 Some conditions affecting the production of gelatinase by *Proteus* bacteria. *J. Bact.* 15: 267-296.
- Munz, E., and Brabender, C. W.
1940 Extensograms as a basis for predicting baking quality and reaction to oxidizing agents. *Cereal Chem.* 17: 313-322.
- Nord, F. F.
1940 Facts and interpretations in the mechanism of alcoholic fermentation. *Chem. Reviews* 26: 423-472.
- Samuel, L. W.
1934 The determination of amino-acids in wheat flour. *Biochem. J.* 28: 273-282.
- Sundstedt, R. M., and Blish, M. J.
1936 Effect of pressure on rate of gas production in yeast fermentation. *Cereal Chem.* 13: 789-790.
- Shen, Ting, and Geddes, W. F.
1942 Protease activity and reducing matter content of wheat flour doughs in relation to baking behavior. *Cereal Chem.* 19: 609-631.

- Sperry, J. A., and Rettger, L. F.
1915 The behavior of bacteria towards purified animal and vegetable proteins.
J. Biol. Chem. 20: 445-459.
- Stephenson, M.
1939 Bacterial metabolism. Longmans, Green and Co., London.
- Werkman, C. H.
1939 Bacterial dissimilation of carbohydrates. Bact. Reviews 3: 187-227.

VISCOSITY CHANGES IN SODIUM SALICYLATE¹ DIS- PERSION OF HARD RED SPRING WHEAT GLUTEN IN RELATION TO VARIETY AND ENVIRONMENT

R. H. HARRIS, RAYMOND V. OLSON, and JOHN JOHNSON, JR.¹

North Dakota Agricultural Experiment Station, Fargo, North Dakota

(Received for publication June 8, 1942)

Harris and Johnson² have previously pointed out differences in the rate at which wheat glutes prepared from different classes and varieties of wheat disperse in 10% sodium salicylate solution. Samples of hard red spring, hard red winter, soft red winter, hard and soft white, and durum wheats belonging to the 1939 crop were examined, and large differences were found in dispersion rates among some classes and varieties. With three varieties of spring wheat grown at three North Dakota stations, curves obtained by plotting viscosity against time of gluten dispersion were similar with one exception. Specific volume of gluten particle (volume occupied by one gram of gluten protein) varied significantly among some of the classes, and was positively correlated with loaf volume. No relationship between these variables appeared within the hard wheat classes.

The effects of such factors as soil and weather upon these gluten properties were not included in the study mentioned above, and no data regarding varietal effects of hard red spring wheats were reported. It was therefore decided to investigate a series of glutes prepared from hard red spring wheats grown in 1939 and 1940 at various locations in North Dakota, with the object of obtaining information on these phases of the problem. Since the paper referred to summarized the literature pertaining to work upon which these researches are based, further reference to previous studies will be omitted.

Experimental Material and Methods

Sixty-eight samples of hard red spring wheat grown at six stations were obtained. These wheats consisted of 13 varieties from repre-

¹At present technologist, Department of Milling Industry, Kansas State College, Manhattan, Kansas.

²R. H. Harris and John Johnson, Jr.: A study of some differences in gluten properties existing between varieties and types of wheat, Cereal Chem. 18: 395-413, 1941.

sentative locations within the state. Twenty were grown in 1939 and 48 in 1940. They were produced under strictly comparable conditions, and all were sound and free from damage that would tend to vitiate the results of study. Some of these varieties have been produced commercially for a number of years, while others are being examined in respect to agronomic and quality performance from year to year in comparison with the older, more "standard" varieties. The samples were cleaned and graded with standard equipment and milled into straight-grade flour on an Allis-Chalmers experimental mill. The temperature and relative humidity of the mill room approximated 70°F and 60% respectively. The flours were baked by the malt-phosphate-bromate method.

The dispersions were prepared by methods outlined in the previous report and consisted, in brief, of mixing to standard consistency by the standard basic formula with 5% sucrose. The gluten was immediately washed from the dough under a standardized stream of 0.1% sodium phosphate at pH 6.8, allowed to stand for a short period under the phosphate solution, then 10 g of the finely divided gluten was added to 100 ml of 10% sodium salicylate solution and dispersed with the aid of constant gentle agitation in an electric rotary shaker. The rate of dispersion was measured in terms of increase in viscosity by withdrawing suitable portions at two-hour intervals, centrifuging, and determining viscosity with a calibrated Ostwald pipette. The liquid and residual gluten were returned to the original flask as soon as possible after completion of the determination. The data were calculated in terms of absolute viscosity, and are presented in the form of curves in which viscosity is plotted against time of dispersion. The relative dispersion rates were also found by calculating the differential quotient dv/dt , where v is the viscosity in centipoises multiplied by 10^3 , and t is the elapsed time in hours. The concentrations of protein in the centrifuged dispersions were determined at the end of 24 hours of dispersion. The specific volumes of the protein particles at this time were also computed, by the formula of Kunitz. Viscosities and dispersion rates were compared at the sixth and eighth hours and comparisons were made of viscosities of the dispersions at 6, 8, and 24 hours.

Results and Discussion

The varieties investigated cover as large a range in strength as it would be possible to find in the hard red spring wheat region. It might have been better if identical stations had been included for the two years, but Fargo and Langdon are common to both years. The wheat protein content varied from 11.6% to 17.5% and the loaf volume from 490 cc to 940 cc. There were marked differences in

viscosity at the 6th hour, but these differences were less noticeable at the 24th hour. The rates of dispersion were particularly variable, ranging from 2.59 to 17.88 after 6 hours of dispersion, whereas following 8 hours they varied from 2.27 to 15.87. These differences were much greater than those found in other parts of the study. Comparatively small differences were evident among the specific volume data.

Thatcher ranked very high in protein content, except at Fargo and Langdon in 1940. Premier, on the other hand, tended to be low in protein content and loaf volume. Their rates of gluten dispersion in sodium salicylate were also very different, Thatcher gluten being much more resistant than Premier. Regent, a comparatively new Canadian variety, also had a resistant gluten. Marquis gluten produced at Fargo and Dickinson in 1940 dispersed very slowly but dispersed more quickly when produced at Langdon. It was intermediate in dispersion rate when grown at Fargo in 1939, resembling Rival, a new variety recently released for distribution in North Dakota, and Vesta.

In Table I are shown average values by station and variety. These data are all from the 1940 crop, and comprise results obtained on 11

TABLE I
STATION AND VARIETY AVERAGES, 1940 CROP

Stations and varieties	Crude protein (N $\times 5.7$) ¹	Flour absorp- tion ¹	Loaf volume ¹	Viscosity		Rate of dispersion		Specific volume
				6 hrs	24 hrs	6 hrs	8 hrs	
		%	%	cc	cp $\times 10^2$		dv/dt	
STATION AVERAGES								
Fargo	15.8	56.4	598	138	246	4.29	5.96	7.18
Dickinson	15.4	57.6	569	159	227	7.86	10.06	6.73
Langdon	14.8	59.9	642	186	236	12.14	11.78	7.09
Mandan	14.2	59.0	602	172	244	9.58	10.70	7.04
VARIETY AVERAGES ²								
Thatcher	15.9	58.3	646	140	239	4.59	6.61	7.01
Pilot B	15.8	57.8	593	176	242	10.53	11.39	7.28
Rival	15.3	58.5	625	162	255	8.28	10.68	7.12
Renown	15.2	57.5	646	155	234	6.85	8.13	7.06
Pilot 13	15.2	58.4	559	156	251	7.26	9.12	7.47
Vesta	15.1	58.6	624	162	244	8.11	9.80	6.87
Merit	15.1	58.4	593	178	225	10.92	11.17	6.61
Premier	14.7	58.1	583	189	226	12.72	12.09	6.55
Marquis	14.4	58.4	599	154	241	6.72	7.74	7.06
Ceres	14.4	58.7	580	172	234	9.78	10.57	7.1
Nordhogen	14.3	57.7	585	158	241	7.42	8.64	7.06

¹ On 13.5% moisture basis.

² Only varieties grown at all four stations are included.

wheat varieties grown at four stations. Two other varieties, grown only at Fargo and Langdon, are not shown in the table. From station averages it is apparent that a difference of 1.6% exists in wheat protein, and a difference of 73 cc in loaf volume. The station with the highest protein content does not show the highest loaf volume, nor

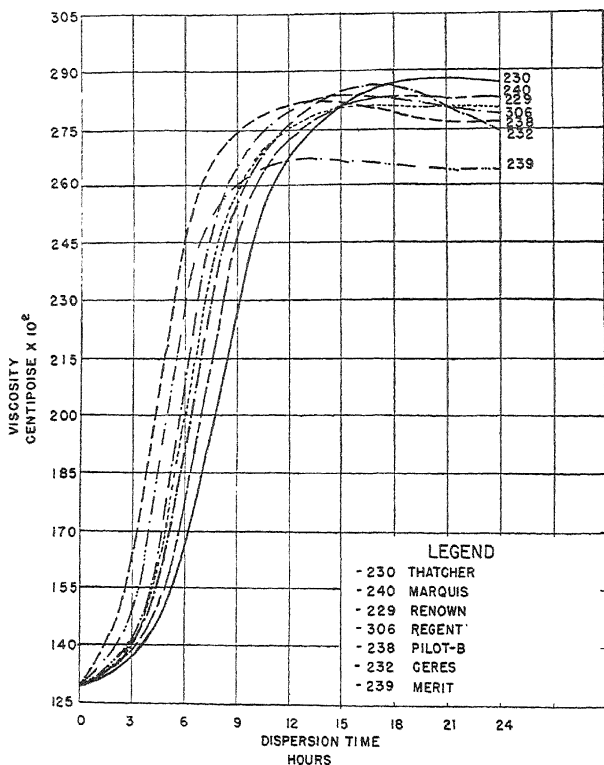


Fig. 1. Dispersion rates of glutes washed from some hard red spring wheat varieties grown at Fargo in 1939.

does the station with the lowest protein content show the lowest loaf volume. The viscosity values at six hours appear to have a tendency to increase as the wheat protein decreases, but no trend is evident after 24 hours of dispersion. Large differences in the rates of dispersion are shown, particularly at the sixth hour. These rates appear to be more or less characteristic of the station and must therefore be connected with environmental factors. The two stations with the highest average wheat protein had the lowest rates but no relation is shown

between these values for the remaining two stations. No marked differences are seen in the specific volume results.

The variety averages have a range of 1.6% in protein and 87 cc in loaf volume. Thatcher was high in both these quality characteristics, but there was little difference between this variety and several

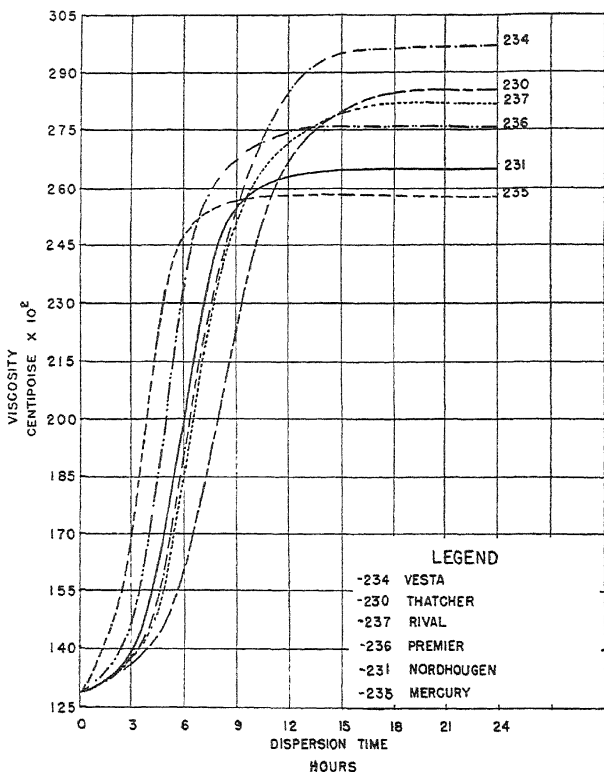


Fig. 2. Dispersion rates of gluts washed from some hard red spring wheat varieties grown at Fargo in 1939.

others. Thatcher had the lowest gluten viscosity in sodium salicylate after six hours, associated with the slowest rate of dispersion. Premier, on the other hand, had the highest gluten viscosity and dispersion rate. The other varieties are spread out between these two extremes, with Renown, Marquis, Pilot 13, and Nordhogen in the higher bracket, while Merit and Pilot B resemble Premier in viscosities and dispersion rates of their gluts. It is evident that wheat variety has a substantial influence upon the rate at which gluten disperses in 10%

sodium salicylate. These characteristic effects of variety and environment are presented graphically by viscosity plotted against time of dispersion.

Figures 1 and 2 show the dispersion rates obtained on glutes prepared from various varieties of hard red spring wheats grown at

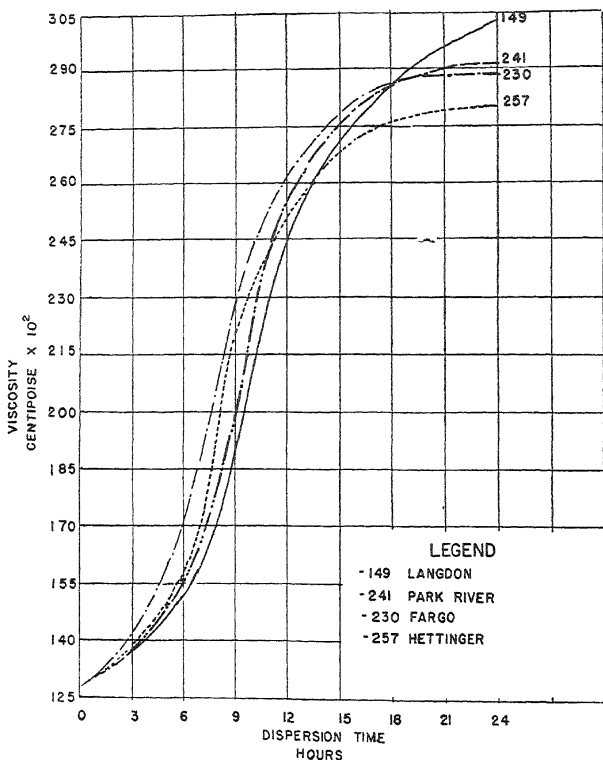


Fig. 3. Dispersion rates of glutes washed from Thatcher wheat grown at four North Dakota stations in 1939.

Fargo in 1939. It was impossible to depict the entire set of curves in one figure, and they were accordingly divided into two sets, Thatcher being included in each for purposes of comparison. In both instances the Thatcher gluten is the most resistant to dispersion. In Figure 1 Marquis follows Thatcher, while Pilot B is least resistant. Merit had the lowest viscosity after 24 hours of dispersion. Regent, Ceres, and Renown glutes have similar dispersion rates. Figure 2 shows greater differentiation than Figure 1 in final viscosities. Mercury gluten dis-

persed most rapidly, with Premier next. Rival, Nordhogen, and Vesta gltens were similar, but Vesta had the highest final viscosity.

In Figure 3 are gluten dispersion rates for Thatcher grown at four North Dakota stations in 1939, while Figure 4 shows similar data for Premier. Apparently no marked effect upon the gluten dispersion

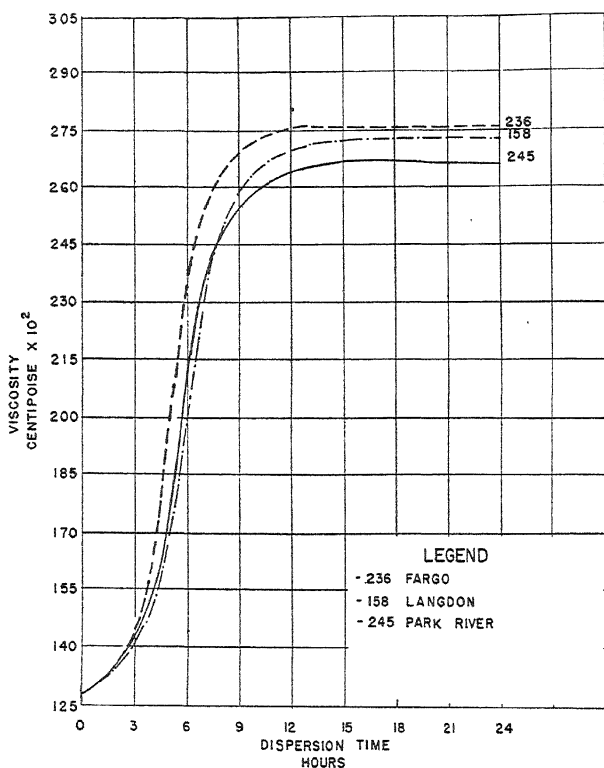


Fig. 4. Dispersion rates of gltens washed from Premier wheat grown at three North Dakota stations.

rate was exerted by environmental differences, and the curve characteristics peculiar to each variety were not greatly affected. In other words, one would conclude that variety was more influential than environment in determining rate of dispersion in 1939, but it must be borne in mind that Thatcher and Premier gltens are very different in dispersibility, and this difference in varietal and environmental effects might not hold for other varieties which are more nearly alike in their dispersion rates.

The data obtained on the 1940-crop wheat glutes are shown in Figures 5 and 6. Figure 5 presents the Fargo sample data, while Figure 6 shows results obtained at Dickinson. Thatcher was included in both figures to afford a basis of comparison. Among the Fargo wheats, Thatcher gluten was the most resistant to dispersion, while

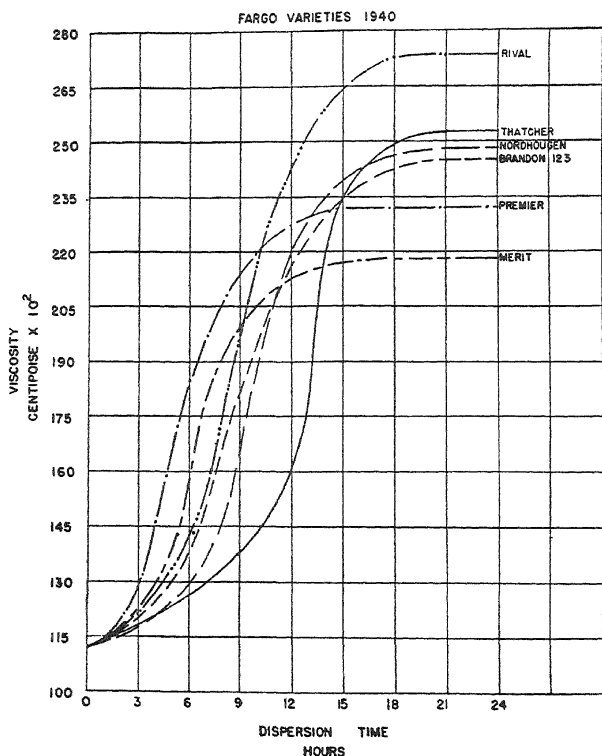


Fig. 5. Dispersion rates of glutes washed from some wheats grown at Fargo in 1940.

Premier was the least. Merit followed Premier in gluten dispersion rate and both varieties had low final viscosities in their dispersions, corresponding to the general trend shown in this figure. Rival gluten was an exception, as it had a medium dispersion rate but exhibited a high viscosity after 24 hours of dispersion. Brandon and Nordhougen glutes had dispersion rates between Thatcher and Rival. The glutes prepared from wheats grown at the Dickinson station showed less differentiation among varieties in respect to dispersion rate than the Fargo wheat glutes. Marquis was the most resistant, with

Thatcher second. Premier gluten dispersed most easily, as in the Fargo group, with Pilot B next in ease of dispersibility. Rival again had the highest final viscosity and Premier the lowest.

Figures 7 and 8 show dispersion data obtained on several varieties grown at different stations in 1940. The stations are presented in

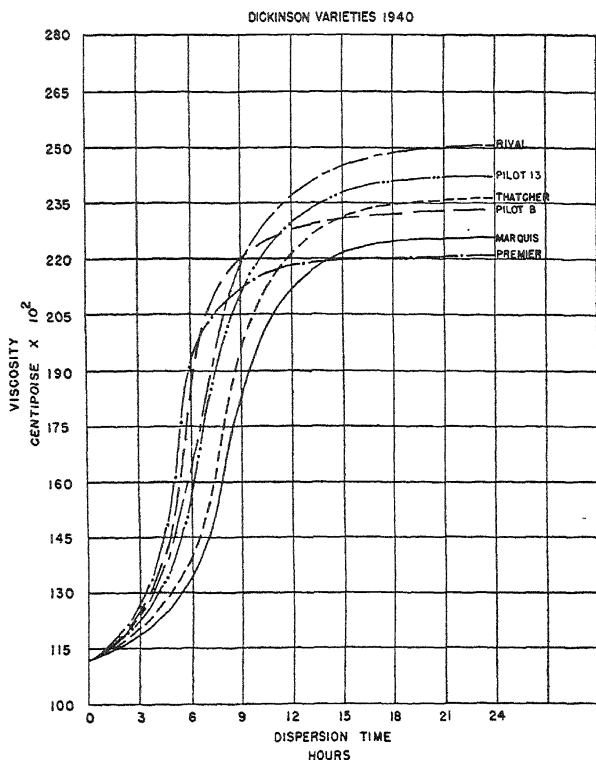


Fig. 6. Dispersion rates of glens washed from some wheats grown at Dickinson in 1940.

groups of three. Mandan and Dickinson also gave somewhat similar curves. Figure 7 shows results from glens prepared from Ceres and Renown grown at Fargo, Langdon, and Dickinson. These varieties were chosen because their curves were best adapted to bring out clearly the differences between stations.

Figure 7 shows distinct differences due to station effects, and emphasizes the greater changes in gluten dispersion rate caused by environmental factors than were apparent in 1939. Langdon glens dispersed the most easily, and were very similar in 24-hour viscosity

to the Dickinson varieties. The situation depicted in Figure 8 is much the same, with Fargo again having the most resistant glutes, Mandan second, and the Langdon samples showing the least resistance for each variety except Rival at Mandan, which dispersed most rapidly of the Rival samples. The varieties arrange themselves in the follow-

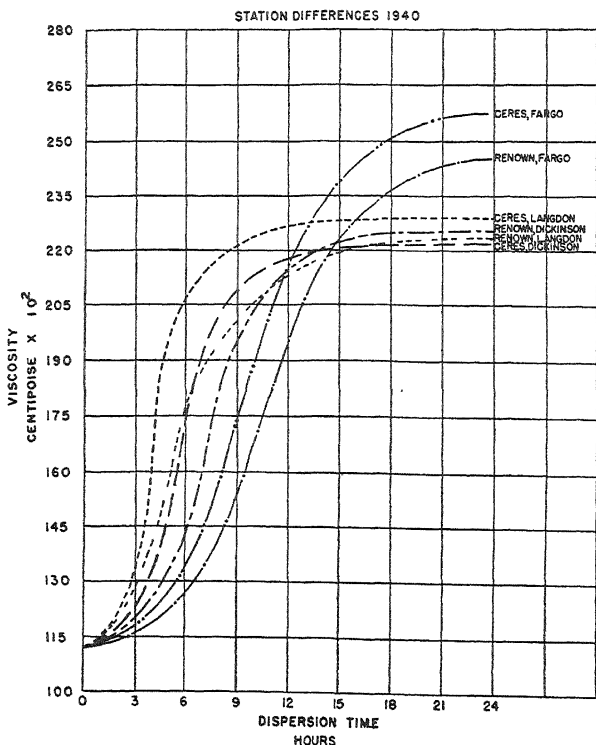


Fig. 7. Comparative gluten dispersion rates of several wheat varieties grown at different stations in 1940.

ing order of gluten dispersibility: Thatcher, Rival, Premier. Rival had the highest final viscosity for each station, while Premier had the lowest. Thatcher was intermediate in every case. The Fargo glutes had the most resistance and, excepting Mandan, Rival also had the highest final viscosity, while Dickinson was intermediate in gluten dispersion rate.

Variations in dispersibility caused by yearly differences are depicted in Figure 9. Three varieties which differed in dispersion rate

are shown. The glutens in this group were washed from wheat varieties grown at Fargo in 1939 and 1940 under comparable plot conditions, except for changes introduced by climatic variability. It is evident that the three varieties fall in the same order of dispersibility each year. It is also apparent that greater differentiation between

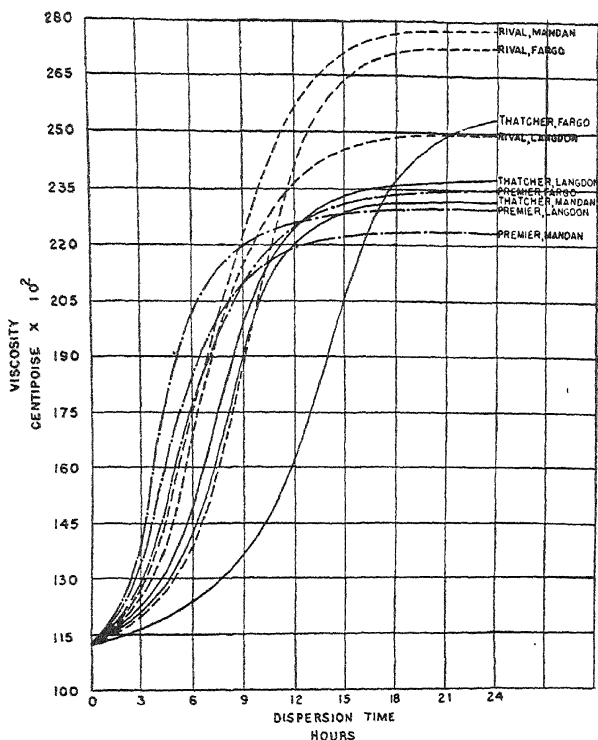


Fig. 8. Comparative gluten dispersion rates of several wheat varieties grown at different stations in 1940.

varieties existed in 1940 than in 1939, while the 24-hour viscosity was slightly higher in 1939. The 1940 glutens tended to be more resistant to dispersion than the corresponding 1939 glutens. These differences were no doubt caused by seasonal variations, and probably are closely related to station differences in these properties.

The results lead to the conclusion that variety has a distinct effect upon the rapidity or ease of dispersion of wheat gluten in 10% sodium salicylate. This varietal effect was shown at different stations where the wheats were grown, and for different crop years. There was also

a marked effect due to station, with Fargo producing the most resistant glutes and Langdon the least resistant, while Mandan and Dickinson were intermediate. These differences appear to vary in magnitude from year to year, and might even be reversed. Wheats grown in different crop years have different dispersion rates, but the

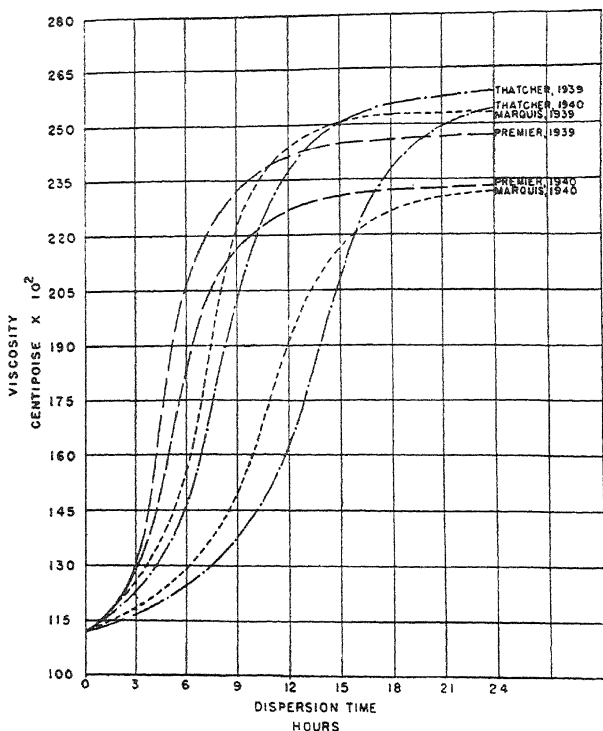


Fig. 9. Differences in gluten dispersion rate due to seasonal variability.

varieties tend to retain their characteristic placings when viscosity is plotted against time.

Table II presents correlation coefficients calculated between the more pertinent variables. Wheat protein and loaf volume are significantly related, but the correlation is too small to be of practical significance for prediction purposes. No doubt the large number of varieties contributed to the low magnitude of this statistic. Wheat protein and rate of dispersion are negatively correlated as would be expected from the observation that very strong wheats, which are usually high in protein, have glutes which tend, on the average, to

be more resistant to dispersion in sodium salicylate, while wheats of lower relative baking strength possess glutens which tend to disperse more rapidly than the average. These latter wheats exhibit trends toward lower protein content.

Specific volume of the protein micelle and loaf volume are not significantly correlated. This result is in agreement with conclusions

TABLE II
CORRELATION COEFFICIENTS COMPUTED FROM THE DATA
(Significant correlation coefficients are in bold type.)

Variables correlated		Correlation coefficients	Probability
<i>x</i>	<i>y</i>	<i>r_{xy}</i>	<i>P</i>
Wheat protein, %	Loaf volume, cc	+.3537	.0038
Wheat protein, %	Rate of dispersion, 6 hrs	-.4002	.0011
Specific volume, ϕ/C	Loaf volume, cc	+.2272	.0635
Rate of dispersion, 6 hrs	Loaf volume, cc	-.0482	>.5485
Viscosity at 6 hrs	Viscosity at 24 hrs	-.2296	.0607
Viscosity at 6 hrs	Viscosity at 8 hrs	+.9028	<.0001

reached by Harris and Johnson (footnote 2) who postulated no correlation between baking quality and specific volume in varieties belonging to the hard wheat classes. Rate of dispersion is apparently not related to loaf volume, as obtained by the baking method used in this investigation. As some of the wheat glutens examined, which dispersed rapidly, had a relatively low viscosity after 24 hours of dispersion, it was thought desirable to determine whether there was a general relationship between these gluten characteristics and, accordingly, the correlation coefficient was computed between viscosities at the 6th and 24th hours. No significant interdependence was found, however.

The relationship between viscosity at 6 and 8 hours is very high and for practical purposes would justify the prediction of one variable from the knowledge of the other. As better differentiation between varieties is shown by the 6-hour results, it would seem that the latter value is of more general utility than the value obtained at any other time.

Because wheat protein and loaf volume, as well as wheat protein and rate of dispersion, are significantly correlated, the partial correlation coefficient between rate of dispersion and loaf volume was calculated, wheat protein being held constant. A value of **+.1081** was obtained, which is below the level of significance.

Although no significant correlation was found between viscosity data and loaf volume, it is felt that a substantial amount of information respecting the gluten properties of the hard red spring wheats studied was gained. Thatcher is universally accepted as a variety

extremely high in strength, with a somewhat harsh, tough gluten, which requires a long mixing time to condition properly. This wheat invariably has a low gluten dispersion rate, usually the lowest of any variety. Premier and Mercury, on the other hand, lack the residual strength found in Thatcher, and their glutes are much less harsh and elastic. These varieties produce glutes which disperse rapidly in sodium salicylate. Other varieties which are intermediate in strength have glutes which disperse more easily than Thatcher but are more resistant than Mercury or Premier. Rival is one of these wheats. It appears probable that hard red spring wheats which possess glutes that disperse comparatively rapidly in sodium salicylate should be viewed with suspicion by the cereal technologist, at least until an extensive study has been made of their baking behavior under varying conditions of formula and method.

TABLE III
ANALYSIS OF VARIANCE OF DISPERSION RATES (DV/DT) OF ELEVEN WHEAT
VARIETIES GROWN AT FOUR STATIONS IN NORTH DAKOTA IN 1940

Source of variation	Sums of squares	Degrees of freedom	Variance	F	5% point	1% point
Between stations	357.554	3	119.185	42.61	2.92	4.54
Between varieties	214.156	10	21.416	7.66	2.16	2.98
Interaction (stations X varieties)	83.906	30	2.797			
Total	655.616	43				

Table III shows the analysis of variance of the rates of dispersion at six hours for the eleven wheat varieties grown at four stations in 1940. In this table the variance calculated for interaction has been used as a criterion for estimating the significance of the variances existing between stations and between varieties. It measures the variation not attributable to either station or variety, its source being in the differential response of the station dispersion rates to wheat varietal trends. If the station averages had varied directly for each variety by precisely the same amount, the resultant interaction would have been zero. Another means of measuring the significance of the two variances would have been to use the differences and sums of duplicate determinations of viscosity, but since these readings were very accurate and were repeated if they differed by more than a set limit, it was thought that the interaction variance would be best to use rather than the variance calculated for the experimental error.

The variance for between stations is very large and gives a high *F* value. The between-varieties variance is smaller, but still extremely significant at both the 5% and 1% points.

The evidence presented in this table fully substantiates the conclusions derived from an inspection of the data given in the tables and figures already discussed.

It is evident that both wheat variety and environmental factors during growth, probably while the gluten proteins are being laid down, have a marked influence upon the gluten complex. This effect is manifested in the comparative rates at which the gluten disperses in 10% sodium salicylate, and is apparently related to certain "quality" characteristics of the gluten. It appears logical to assume that the structure of the complex has been altered in some manner by these influences and this change is reflected in the ease with which the gluten particles are dispersed. It is probable that changes take place in the manner in which the constituent portions of the complex are united. Wheats whose glutes disperse easily would have the constituent proteins less rigidly held together than in the instance of wheats possessing glutes which are resistant to dispersion. These differences in constitution would be shown in certain properties which are concerned with the "feel" of the gluten during washing, and in reactions of the gluten during the baking process. Factors which favor high wheat protein content have a tendency to increase gluten resistance to dispersion, probably by increasing the tenacity of bonds within the gluten complex. These changes do not affect the size, or hydration, of the gluten micelle to any marked degree in hard red spring wheats.

Summary and Conclusions

An investigation conducted upon 68 samples of hard red spring wheat, comprising 13 varieties grown at 6 North Dakota stations in 1939 and 1940, appears to justify the following conclusions:

The rate at which gluten washed from hard red spring wheat flour disperses in 10% sodium salicylate solution is related to wheat variety.

This dispersion rate is also related to the environmental conditions of soil and climate under which the wheat is grown, and accordingly the different stations produce wheats having significantly different rates.

A corollary to the statement respecting differences induced by environmental variations is that wheats grown in different years have glutes which disperse at different rates.

The differential quotient dv/dt appears to have the more significant value at the 6th hour of dispersion, owing to changes in the rates occurring at later periods of the dispersion process. In some instances the rate begins to fall off.

The specific volume ϕ/C of the gluten micelles in sodium salicylate dispersion is not significantly correlated with wheat protein content or loaf volume in hard red spring wheat varieties. This conclusion is in agreement with the hypothesis of Harris and Johnson (footnote 2), who found a significant relationship between specific volume and loaf volume when a number of wheats belonging to different classes were examined, but postulated no correlation within classes.

Glutens prepared from hard red spring wheats of high protein content tend to disperse more slowly than wheats of lower protein content.

Despite lack of relationship between the viscosity data and baking strength as registered by loaf volume, it is felt that valuable information respecting varietal and environmental gluten differences was obtained by determining the changes in viscosity of wheat gluten dispersion in 10% sodium salicylate between 0 and 6 hours of dispersion time.

Acknowledgments

The authors wish to acknowledge the valuable contributions made by L. D. Sibbitt in conducting the milling and baking determinations required in this investigation. Credit is also due to T. E. Stoa, Station Agronomist, who furnished the wheat samples, as well as to the NYA, and WPA Seed Testing Project, O.P. 165-1-73-144, for technical assistance.

THE COMPARATIVE BAKING QUALITIES OF HARD RED SPRING WHEAT STARCHES AND GLUTENS AS PREPARED BY THE GLUTEN-STARCH BLEND BAKING METHOD¹

R. H. HARRIS and L. D. SIBBITT

North Dakota Agricultural Experiment Station, Fargo, North Dakota

(Received for publication September 29, 1941)

A number of papers have been published recently dealing with various methods of adding wheat gluten and starch to flour, or of mixing these constituents with each other, to form blends at a uniform protein level. These blends were then baked and the loaves examined in respect to comparative baking quality. By this method differences in baking strength caused by variations in protein quantity would be ruled out.

Aitken and Geddes (1938) raised the gluten content of a series of wheat flours by the addition of dried ground gluten and baked the doughs made therefrom. The resultant loaves closely resembled those

¹ Published with the approval of the Director of the Station.

produced by flours of corresponding natural protein content. The glutens were prepared from different wheat varieties and classes. With the exception of some very weak wheats (such as German and English) the gluten quality appeared to be fairly constant.

The senior author, in collaboration with H. N. Bergsteinsson,² built up the protein content of a soft-wheat flour dough by incorporating freshly washed wheat gluten in the dough mix. Marked improvement in loaf volume, external appearance, and internal characteristics were caused by the addition of the gluten.

Later Harris (1940) employed a method essentially resembling the procedure of Aitken and Geddes, wherein dried ground gluten was added to a series of flours milled from hard red spring, hard red winter, soft red winter, and durum wheats. The blends were then baked at a constant protein level. Hard-wheat glutens improved the loaf volume of the soft-wheat base flour used, while durum gluten tended to decrease the loaf volume. The average color and texture scores were raised by the addition of the dried gluten. The malt-phosphate-bromate baking formula was used in the three investigations mentioned above.

Sandstedt, Jolitz, and Blish (1939) showed the feasibility of baking bread from blends of dried gluten and starch prepared from wheat flour. Satisfactory loaves were produced, and the authors stated that the blend loaves closely resembled those baked from the original flour. Harris and Sibbitt (1941) applied this method to the determination of the baking quality of a series of starches prepared from different classes and varieties of wheat. A common gluten substrate washed from hard red spring wheat was used and suitable proportions of starch were then superimposed upon it. Three protein levels (10.0%, 13.2%, and 16.0%) were employed. The results of this investigation showed that starches prepared from different wheats produced loaves differing greatly in volume, general appearance, and internal characteristics.

Sandstedt and Ofelt (1940) diluted a series of hard red winter wheat flours with starch prepared by the method used by Sandstedt, Jolitz, and Blish (1939). A constant protein level of 10.0% was used, and the diluted flours were baked by a series of formulas. The data obtained indicated great variability in protein quality among varieties. Within varieties protein quality also varied with the original wheat protein content. Fortmann and Sandstedt³ extended this series of protein quality investigations. Ten wheat varieties grown at 14 Nebraska locations were examined. It was found that any one variety

² At present, research chemist, Lake of the Woods Milling Company, Keewatin, Ontario.

³ Karl Fortmann and R. M. Sandstedt: Effect of environment and variety on wheat flour quality. Read at A. A. C. C. meeting in Omaha, 1941.

grown in different localities showed striking differences in flour quality. It was also shown that varieties grown in any particular environment had much the same relative baking qualities in reference to each other as the same varieties had when grown under other environments. It is essential when evaluating varieties that they be grown under identical conditions.

In view of the results obtained in the investigations cited, it was thought desirable to study the effects of wheat variety and location of growth upon the baking results obtained from a series of starch-gluten blends made from hard red spring wheat starch and gluten. In this way the influences of variety and locality upon wheat starch and gluten could be examined separately by blending starches prepared from various varieties of commercial importance with a constant gluten substrate, and then reversing this plan and baking different glutes with a constant starch.

Experimental Material and Methods

Forty-eight samples of starch were prepared from 13 varieties of hard red spring wheat by a method which was essentially that used by Sandstedt, Jolitz, and Blish (1939). Eleven of these varieties were grown in replicated plots under comparable conditions at four different stations in North Dakota in 1940. The remaining two varieties were grown at only two stations. They were free from any damage that might tend to influence the results with respect to varietal or environmental differences. The wheats were milled into straight-grade flour on an Allis-Chalmers mill under laboratory conditions of approximately 70°F temperature and 60% relative humidity. The glutes washed from these flours were divided into pieces averaging $\frac{1}{2}$ g in weight, placed on waxed paper sheets, and dried at 90°F for 24 hours. During the first 8 hours the relative humidity of the drying cabinet was gradually reduced from 94% to room conditions (approximately 50%). The sheets were hung parallel to the air current in the drier. The dry gluten pellets were removed from the sheets and reduced to a powder in a suitable grinder.

The starches and glutes were analyzed for moisture and total protein content. From the data the respective proportions of the two ingredients required to furnish a protein content of 13.2% on a 13.5% moisture basis were calculated. The procedure employed in mixing the blends was to hydrate the gluten for one hour by the addition of a constant volume (12 ml) of distilled water at 30°C, and then add the hydrated gluten plus any unabsorbed water to the dry starch in the mixer bowl. The Hobart mixer with two dough hooks was used to mix the doughs. A total mixing time of $2\frac{1}{2}$ minutes minus 15 seconds

for scraping down the pins and sides of the bowl was used. The formula was the malt-phosphate-bromate with 7% sucrose. The micro baking procedure was used with standard fermentation periods, as described by a number of investigators (Geddes and Aitken, 1935; Harris and Sanderson, 1938; VanScoyk, 1937, 1939). The loaf volumes were determined 30 minutes after baking by a calibrated micro loaf volumeter. The original flours were baked by the 100-g method using the Hobart-Swanson and the malt-phosphate-bromate formula. The morning after baking, all loaves were examined under a fluorescent light for crust color, external appearance, and internal characteristics.

Discussion

The varieties included in the study were those of particular interest to the northwestern hard red spring wheat area, comprising several of the older wheats which have been grown in this region until they have come to be considered more or less as standards with which newer varieties may be compared in respect to agronomic and quality factors. Included also were some wheats which have been recently released for growth within the state, as well as a number that are being tested with a view to later distribution. Members of the first group, the older tried wheats, are Marquis, Ceres, and Thatcher, while varieties that have been recently introduced into the variety picture are Pilot B, Rival, and Nordhougen.⁴ Wheats under trial are Merit, Pilot 13, Vesta, and Premier. The remaining varieties are those which have originated in Canada and may be grown to some extent in the northwestern states if they prove satisfactory.

The four stations at which the wheats were grown are located in the eastern, central, western, and northeastern sections of the state and fairly well cover the principal portion of the wheat-producing area of North Dakota.

Some differences in test weight per bushel were evident in the data, but these are not more than would be expected where a number of wheat varieties are grown under variable environmental conditions. The wheat protein ranged from 13.0% to 16.6%, and the flour yield from 60.2% to 75.7%. The ash values were normal for experimental flours milled from hard red spring wheat varieties. Some variability was evident in the starch and gluten moistures but these differences were not related to wheat variety. It was found that moisture content varied more among starches than among glutens, a result in all probability of more constant conditions when the latter material was dried. The starch moisture ranged from 6.7% to 12.1%, while the gluten varied from 8.3% to 10.6%. The protein content of the

⁴ This wheat was developed by a private grower and is being produced to some extent in the state.

starches was quite uniform, differing by not more than 0.2%, but the protein content of the dried glutens varied from 63.5% to 75.5%. The glutens washed from wheats grown at the Dickinson Station were highest in protein content.

The comparative baking data obtained on the original flours and the starch-gluten and gluten-starch blends led to the following conclusions. The absorption of the blends was higher owing principally to their lower moisture content as compared with the original flours. The loaf volumes obtained on the flours cannot be directly compared with the corresponding values of the blends owing to the facts that the protein contents were different in the original flours and a different baking method was used for the flours than was employed for the blends. The flour absorptions and loaf volumes showed marked variability. The absorption range was from 55.3% to 60.7% and the loaf volume range from 525 cc to 720 cc. There were also substantial differences in crumb color scores, which varied from 6.2 to 8.5.

TABLE I
STATISTICAL CONSTANTS COMPUTED FROM THE DATA

Variable	Mean	Minimum	Maximum	Range	Standard deviation	Coefficient of variability
Absorption, original flours, %	58.2	55.3	60.7	5.4	1.507	2.59
Absorption, starch-gluten blends, %	68.3	66.6	70.6	4.0	1.119	1.64
Absorption, gluten-starch blends, %	64.3	61.6	67.6	6.0	1.355	2.11
Loaf volume, starch-gluten blends, cc.	131.0	114.0	145.0	31.0	8.633	6.59
Loaf volume, gluten-starch blends, cc	131.3	106.0	160.0	54.0	11.939	9.09
Crumb color, original flours	7.4	6.2	8.5	2.3	0.598	8.08
Crumb color, starch-gluten blends	7.0	6.5	7.5	1.0	0.343	4.90
Crumb color, gluten-starch blends	7.1	5.5	8.0	2.5	0.769	10.83

The absorptions in the starch-gluten blends were less variable than in the case of the original flours and ranged from 66.6% to 70.6%, a difference of 4% as compared with 5.4% for the flours. The loaf volumes ranged from 114 cc to 145 cc. The crumb colors varied from 6.5 to 7.5, a very small difference. It was evident that loaves baked from the starch-gluten blends, prepared in the manner described, do not show the differences in characteristics to be found among the loaves baked from the original flours themselves. This result is to be expected, since some of the sources of variability have been removed. The protein content is uniform, while a constant gluten substrate has been employed throughout the series, thus obviating differences due to possible variations in gluten quality. Other causes of difference may be found in the removal of certain flour constituents, as enzymes,

lipids, inorganic salts, etc., during the washing of the starch and gluten. Despite the removal of certain proportions of these constituents, however, there can be little doubt that differences in results have been caused by inherent variability in the starches themselves. This variability may have been due to factors such as differences in starch damage during milling, or to other causes.

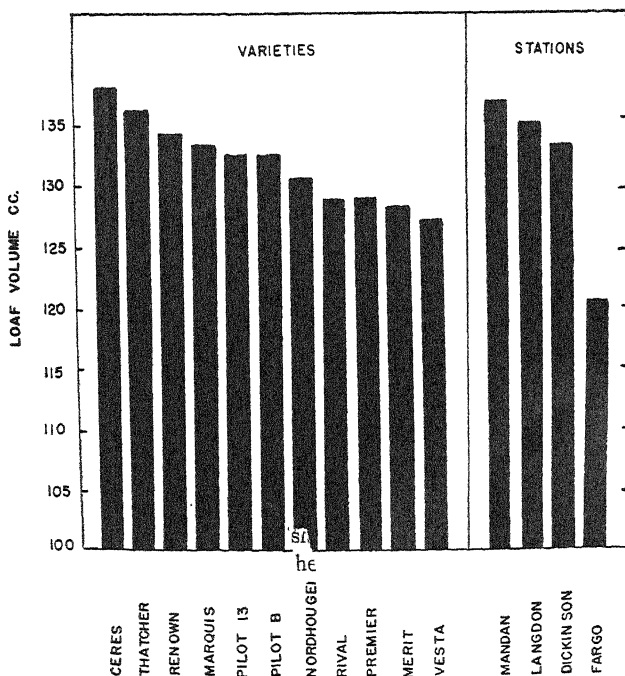


Fig. 1. Comparative loaf volumes obtained by baking blends of starch with a common hard red spring gluten substrate.

The absorption values of the blends made with a variable gluten superimposed upon a constant starch base were lower than the starch-gluten values, but higher than those of the flours themselves. They varied from 61.6% to 67.6%, a difference of 6.0%, as compared to 5.4% for the flours and 4.0% for the starch-gluten doughs. The loaf volumes ranged from 106 to 160 cc, a difference of 54 cc, while there was a difference of only 31 cc in the starch-gluten blends. The crumb colors varied from 5.5 to 8.0, or a difference of 2.5 as contrasted with 1.0 for the starch-gluten loaves and 2.3 for the original flours. These comparative results are presented in condensed form in Table I, which

gives means, minima and maxima, ranges, standard deviations, and coefficients of variability for the different sets of baking data.

It is apparent that the mean loaf-volume values for both series of blends were the same. There was also more variability in loaf volume in the gluten-starch than in the starch-gluten blends. The crumb color

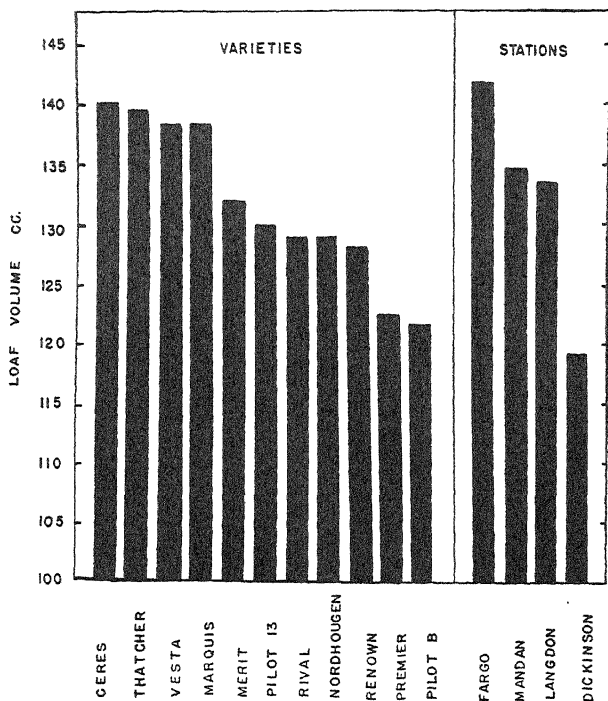


Fig. 2. Comparative loaf volumes obtained by baking blends of gluten with a common hard red spring starch substrate.

in the gluten-starch blends was more variable than in the other two series of bakings, while it was least variable in the starch-gluten blends.

For the purpose of making comparisons of starch and gluten baking qualities among wheat varieties and stations, the loaf volume data from the eleven varieties which were grown at all the stations were next examined. The comparative loaf volumes obtained on the starch-gluten blends are shown in Figure 1. The data are arranged in order of decreasing loaf volume from left to right. Ceres wheat starch produced the largest loaf in this series, while Thatcher starch gave the next largest. Rival, Premier, Merit, and Vesta starches gave the

lowest loaf volumes. Both varieties of Pilot were equal in starch baking quality and are in the same range as Marquis starch. Variation in mean loaf volumes among stations was more pronounced than among varieties, Fargo being substantially below the other three stations, which gave very similar results.

Figure 2 shows similar data for the gluten-starch blends. Larger differences among varieties are apparent than were found in the case of the starch-gluten blends. Ceres, Thatcher, Vesta, and Marquis are similar in results obtained by baking their glutens with starch as

TABLE II
ANALYSIS OF LOAF-VOLUME DATA

Source of variance	Sum of squares	Degrees of freedom	Variance	F	5% point	1% point
ANALYSIS OF VARIANCE OF STARCH-GLUTEN LOAF VOLUMES						
Between stations.....	1814.97	3	604.99	25.84	2.92	4.51
Between varieties.....	551.00	10	55.10	2.35	2.16	2.98
Interaction (stations \times varieties).....	702.28	30	23.41			
Total.....	3068.25	43				
ANALYSIS OF VARIANCE OF GLUTEN-STARCH LOAF VOLUMES						
Between stations.....	2910.91	3	970.30	14.92	2.92	4.51
Between varieties.....	1611.23	10	161.12	2.48	2.16	2.98
Interaction (stations \times varieties).....	1950.59	30	65.02			
Total.....	6472.73	43				

described. An intermediate group consists of Merit, Pilot 13, Rival, Nordhousen, and Renown, while Premier and Pilot B fall into the lowest group. There is a substantial difference between the mean blend loaf volumes for Dickinson and the other three stations. In this series of bakings the Fargo wheats yielded the largest loaves, while Mandan and Langdon were the same.

The data from the two series of blends were analyzed to determine the significance of the differences of the mean blend loaf volumes among the various varieties and stations. The results obtained from the two series of blends are shown in Table II. The variance for interaction (stations \times varieties) was used to measure the significance of the variances due to station and variety differences. The interaction variance arises from the differential response of the station loaf volumes to the varietal trends.

The significance of the differences between station and variety

means may be determined by the use of the standard error s .

$s = \sqrt{23.409} = 4.838$ in the case of the starch-gluten blends.

Then $s_m = 4.838/\sqrt{4} = 2.419$, and the standard error of the difference between the means of any two varieties is then $2.419 \times \sqrt{2} = 3.42$. For Thatcher and Vesta, the difference between means is 9.0 cc. Therefore, $t = 9.0/3.42 = 2.63$. This is a significant difference inasmuch as t at the 5% point is 2.04.

To determine the significance of the difference between station means the same technique is used, except that s_m is now $4.838/\sqrt{11} = 1.458$, as there were 11 varieties grown at each station.

The significance of the differences in mean gluten-starch loaf volumes may be found in a similar manner.

From the results enumerated it would appear that the chief source of variability in baking quality when the protein content is held constant lies in the properties or "quality" of the gluten proteins. This conclusion is in agreement with the general consensus of opinion among wheat technologists and corresponds with the postulate of Sandstedt and Ofelt (1940) and Fortmann and Sandstedt (footnote 3). Harris and Sibbitt (1941) have indicated that very substantial differences in baking results may be brought about by varietal effects upon starches used in a baking blend with constant gluten menstrooms. Their work was done with wheats belonging to different classes, however, and this fact would partly account, no doubt, for the larger differences in starch baking quality which they found.

Summary and Conclusions

Forty-eight samples of starch and gluten were prepared essentially by the procedure reported by Sandstedt, Jolitz, and Blish (1939) from 13 varieties of hard red spring wheat grown at four stations in North Dakota. The starches were blended individually with a common gluten substrate prepared from hard red spring wheat, and made into doughs and baked. Similarly, the different glutes were blended with a hard red spring wheat starch and baked. These two series of bakings were done at a constant protein level. The standard basic formula with 7% sucrose was employed, with the micro baking method. The results obtained appeared to justify the following conclusions:

Doughs made from starch-gluten blends are higher in absorption than the original flour doughs. This is caused largely by the lower moisture level of these four constituents after drying. The absorption of doughs made from a variable starch with constant gluten was higher than that of doughs made with a variable gluten and constant starch. The reason for this difference is not clear.

The crumb-color score of the bread made from the original flours was higher than the blend-loaf score.

Significant differences in loaf volume of the blends were shown between varieties of wheat, as well as between locations of growth, by the analysis of variance. It would follow that both the starches and glutens prepared from hard red spring wheats by the method used in this study vary in baking quality as a result of both inherited and environmental factors. These differences, as far as variety is concerned, tend to show that certain wheats which have been viewed with suspicion in respect to baking strength have glutens which yield loaves of inferior volume as compared to glutens washed from wheats commonly considered to be of superior baking strength. Some of these superior wheats apparently have starches which produce loaves of relatively high quality when used in starch-gluten blends.

Because of the small number of samples of each variety included in the study it was not possible to draw conclusions regarding the effect of original wheat protein content upon gluten or starch baking strength within a variety.

Literature Cited

- Aitken, T. R., and Geddes, W. F.
1938 The effect on flour strength of increasing the protein content by addition of dried gluten. *Cereal Chem.* **15**: 181-196.
- Geddes, W. F., and Aitken, T. R.
1935 An experimental milling and baking technique requiring 100 grams wheat. *Cereal Chem.* **12**: 696-707.
- Harris, R. H.
1940 A comparative study of some properties of dried glutens prepared from various types of wheat. *Cereal Chem.* **17**: 222-232.
- and Sanderson, T.
1938 A comparison between the 100 and 25 gram baking methods. *Cereal Chem.* **15**: 251-256.
- and Sibbitt, L. D.
1941 The comparative baking qualities of starches prepared from different wheat varieties. *Cereal Chem.* **18**: 585-604.
- Sandstedt, R. M., Jolitz, C. E., and Blish, M. J.
1939 Starch in relation to some baking properties of flour. *Cereal Chem.* **16**: 780-792.
- and Ofelt, C. W.
1940 A varietal study of the relation between protein quality and protein content. *Cereal Chem.* **17**: 714-725.
- VanScoyk, W. V.
1937 A mold for micro-baking. *Cereal Chem.* **14**: 263-265.
1939 Micro-baking technique, application and results. *Cereal Chem.* **16**: 1-12.

THE EFFECT OF TEMPERATURE UPON THE VIABILITY AND BAKING PROPERTIES OF DRY AND MOIST YEAST STORED FOR VARIED PERIODS

E. J. THIESSEN

Wyoming Agricultural Experiment Station, Laramie, Wyoming

(Received for publication May 11, 1942)

It frequently becomes necessary to store yeast in small or large quantities for a time before use, particularly in isolated parts of the country. The best methods for the preservation of yeast, as with other perishable products, are of especial importance in the present war program when transportation facilities are being curtailed.

A review of the literature reveals that considerable experimental testing has been carried on with regard to the viability of different brands of moist yeast, both fresh and stored. No published material was found on the viability of dry yeasts, which are commonly used for home baking in rural areas.

Iwanowski and Brezezinski (1934) found that moist yeast could be stored at 32°F for two to three months without much loss in activity, whereas 10% to 20% of the cells were dead in yeast stored at 56°F for about two weeks and 20% when stored at 72°F for one week.

Bohn and Favor (1939) compared five brands of moist yeast by means of the pressuremeter and found variations in the amount of gas liberated from hour to hour by the different brands. Doty and Urban (1940) correlated the rate of gas production in moist yeast with the texture and crust characteristics of bread. The quantity of gas produced during the fourth hour gave a good picture of what the yeasts would do in a dough.

Weaver, Talbott, and Coleman (1933) reported that during aging there was a marked change in the general appearance of moist yeast. One brand retained its leavening qualities without impairment for 96 hours, with only slight changes at the end of 168 hours. Bread from the other brand showed a marked decrease in loaf volume, grain, texture, and crumb color scores after the yeast was stored 48 hours.

Bailey, Bartrom, and Rowe (1940) made bread with frozen moist yeast. They reported that the temperature of evaporated dry ice (-109°F) injures yeast in 24 hours and subsequent storage below freezing increases the amount of deterioration. The most satisfactory storage temperature was reported as 30°F, although comparatively good bread was made with frozen yeast stored a month or two at 20°F.

Experimental Procedure

In the experiments reported in this study, the viabilities of both dry and compressed yeast have been investigated. Dry yeasts secured from the factory were separated into lots and placed in airtight glass containers. A part of each was stored in the refrigerator with temperatures ranging from 40° to 50°F and a part at room temperatures of 70° to 75°F. Compressed yeast was tested fresh and frozen. The frozen yeast was stored from one to twelve months. The dry yeast was stored for as long as 32 months.

The Sandstedt-Blish pressuremeter was utilized to measure periodically the gas produced by these yeasts in a dough. The formula with compressed and quick-action dry yeast consisted of 0.3 g yeast (3% based on flour), 0.574 g sucrose, 10 ml water, and 10 g flour. The formula with dry yeast cakes, which required a considerably longer fermentation for an equal degree of lightness, consisted of 0.6 g yeast, 0.574 g sucrose, 2 g mashed potatoes, 8 g flour, and 10 ml water. Several formulas were tested with the fresh dry cakes in order that the fermentation could be speeded up sufficiently to secure, over an eight-hour period, pressuremeter readings which approximated those secured with moist yeast in a dough fermented four hours. Doubling the amount of yeast and the addition of mashed potatoes aided in this. Potatoes are quite generally used in home baking with dry yeast cakes.

The quantity of sugar necessary was calculated according to the method of Sandstedt (private correspondence) by the formula $1000 - (4/3 \text{ gassing power}) = \text{milligrams of sugar to be added}$, $4/3$ gassing power being the quantity of sugar developed by diastasis in the flour in the course of a four-hour fermentation. In the recorded tests with the various yeasts, one flour was used for the doughs.

The fermentation was carried on at a constant temperature of 86°F. Readings were recorded and the gas in the pressuremeter was released at the end of each hour. The gas was allowed to escape slowly, so that the cooling effect due to adiabatic expansion would not affect the readings.

The viability of the moist yeast was further tested by staining with methylene blue, which stains the dead and injured but not the living cells.

Results

Quick-action dry yeast: The pressuremeter readings during the fermentation of the quick-action dry granulated yeast stored under the conditions noted are given in Table I. It is evident from the table that the loss of activity was considerably greater for this yeast

TABLE I

PRESSUREMETER READINGS DURING FERMENTATION OF QUICK-ACTION DRY YEAST STORED FOR VARIOUS PERIODS OF TIME AND AT DIFFERENT TEMPERATURES

Quick-action dry yeast stored at 40°-50°F			Quick-action dry yeast stored at 70°-80°F		
Time in storage	Gas	Loss in activity	Time in storage	Gas	Loss in activity
	<i>mm Hg</i>	<i>%</i>		<i>mm Hg</i>	<i>%</i>
Fresh	810	—	Fresh	810	—
1 month	770	4.9	1 month	720	9.9
6 months	625	22.8	7 months	510	37.0
12 months	520	35.8	12 months	390	51.9
31 months	425	47.5	18 months	230	71.6

stored at room temperature than for the same lot stored in the refrigerator, and also that its activity became consistently less as the time in storage was prolonged. Differences in the quality of the bread made with quick-action dry yeast stored at different temperatures for varied times are shown in Figure 1.

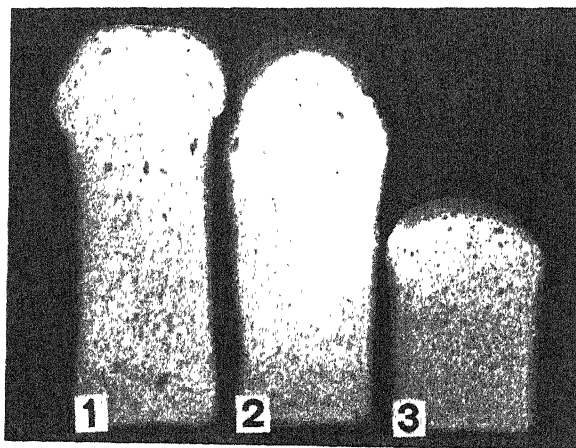


Fig. 1. Loaves made from quick-action dry yeast. Loaf No. 1 was made with the fresh yeast. Loaf No. 2 with yeast stored in the refrigerator at 40°-50°F for 12 months, and loaf No. 3 from the same lot of yeast stored at a room temperature of 70°-80°F.

In Figure 1, loaves 2 and 3 were made from the same lot of yeast, but for loaf No. 2 the yeast was stored at 40° to 50°F, while for No. 3 it had been stored at room temperature for twelve months. The extremely poor quality of loaf No. 3 as compared with No. 2 makes it evident that the length of time this yeast can be stored satisfactorily depends in a large measure upon the temperature of storage.

Each loaf shown in Figure 1 contained 2 g of quick-action dry yeast and 340 g of flour, together with sugar, salt, fat, and water in weighed amounts. The total fermentation of the dough of loaf No. 3 was 50 minutes longer in an attempt to lighten it sufficiently for baking.

Dry yeast cakes: The activity of dry yeast cakes, used with the formula specified and fermented in the pressuremeter for eight hours, is recorded in Table II. This type of yeast is rapidly being replaced

TABLE II

PRESSUREMETER READINGS DURING THE FERMENTATION OF DRY YEAST CAKES STORED FOR VARIED PERIODS OF TIME AND AT DIFFERENT TEMPERATURES

Dry yeast cakes stored at 40°-50°F			Dry yeast cakes stored at 70°-85°F		
Time in storage	Gas	Loss in activity	Time in storage	Gas	Loss in activity
	<i>mm Hg</i>	<i>%</i>		<i>mm Hg</i>	<i>%</i>
3 days	552	—	3 days	552	—
4 months	540	2.2	4½ months	230	58.3
10 months	338	20.6	6 months	185	66.5
15 months	260	52.9	8 months	90	83.7
32 months	185	66.5	17½ months	25	95.4

by those of speedier action. It is, however, still used to a considerable extent for home baking in rural districts. The superiority of cold temperatures for the preservation of dry yeast cakes is strikingly evident, as can be noted from the data and from the bread baked from this yeast as shown in Figure 2.

In Figure 2, loaves No. 1 and No. 2 became light with almost equal speed in the dough but No. 3 was fermented 2½ hours longer in an attempt to increase its lightness sufficiently for baking. With other lots the loss varied somewhat from the above figures but followed the same trend. Each loaf contained 4 g of the dry yeast cake, 15 g of mashed potato, and 100 g of flour fermented in a sponge over night in a proofing cabinet at 70°F. An additional 240 g of flour together with fat, sugar, salt, and water in weighed amounts was added the following morning. The dough was fermented and pan proofed as with the quick-action yeasts. The length of time the dry yeast cakes can be stored and retain sufficient viability for good bread depends upon the temperature of storage.

Moist yeast: The viability of three lots of moist yeast in a fresh and frozen condition was measured.

It has long been recognized that cold temperatures are essential to keep moist yeast in good condition. Bailey, Bartram, and Rowe (1940) reported that temperatures slightly above the freezing point

or about 30°F were satisfactory for continued storage. It is conceivable that this temperature might be difficult to maintain when yeast is transported or stored at home. The temperatures in household mechanical refrigerators are usually maintained around 40°-50°F for ordinary refrigeration. At such temperatures moist yeast soon becomes discolored and can be preserved for only short periods.

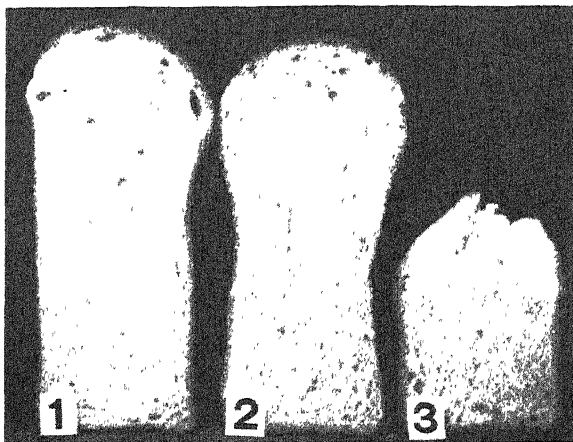


Fig. 2. Loaves made from slow-action dry yeast cakes. Loaf No. 1 was made from the fresh dry cake, loaf No. 2 from a cake stored in the refrigerator at 40°-50°F for 12 months, and loaf No. 3 from the same lot of yeast stored at a room temperature of 70°-80°F.

Freezing was considered the most convenient method for the storage of moist yeast in isolated districts and was therefore used in these tests. Frozen moist yeast may be conveniently stored in freezer lockers in rural districts. The freezing units in household refrigerators may be utilized for short storage periods. Precautions must be taken that the yeast is not allowed to thaw during the storage time. The activity of the frozen yeast stored for varied periods at 19°F, the temperature at which the home refrigerator freezing unit is often kept, was measured in the pressuremeter and is recorded in Table III.

It is evident from Table III that the compressed yeast frozen for a month liberated almost a normal amount of gas with surprisingly slight losses after being frozen three or four months. Its activity was measured by testing portions of the same cake from each of the above lots after these varied storage periods. Yeast from these lots was also tested in bread from time to time.

All test loaves made with moist yeast contained 7 g of yeast and 340 g of flour, together with fat, sugar, salt, and water in weighed

TABLE III
PRESSUREMETER READINGS ON THREE LOTS OF FROZEN COMPRESSED YEAST
STORED FOR VARIED PERIODS

Lot I			Lot II			Lot III		
Time kept frozen	Gas	Loss in activity	Time kept frozen	Gas	Loss in activity	Time kept frozen	Gas	Loss in activity
	<i>mm Hg</i>	<i>%</i>		<i>mm Hg</i>	<i>%</i>		<i>mm Hg</i>	<i>%</i>
Fresh	500	—	Fresh	505	—	Fresh	500	—
4 wks	495	1.0	8 wks	500	1.0	4 wks	497	0.6
8 wks	490	2.0	12 wks	490	3.0	10 wks	493	1.4
12 wks	490	2.0	20 wks	475	5.9	13 wks	493	1.4
16 wks	475	5.0	26 wks	385	23.8	18 wks	492	1.6
24 wks	410	18.0				24 wks	420	16.0
40 wks	340	32.0	44 wks	320	36.6	48 wks	180	64.0
48 wks	320	36.0						

amounts. The fermentation was carried on in a proofing cabinet with temperatures constant. The total time required for fermentation and pan proofing of the dough made from one of the lots of yeast frozen for a month was approximately 20 minutes more than was necessary to reach the same degree of lightness with fresh yeast.

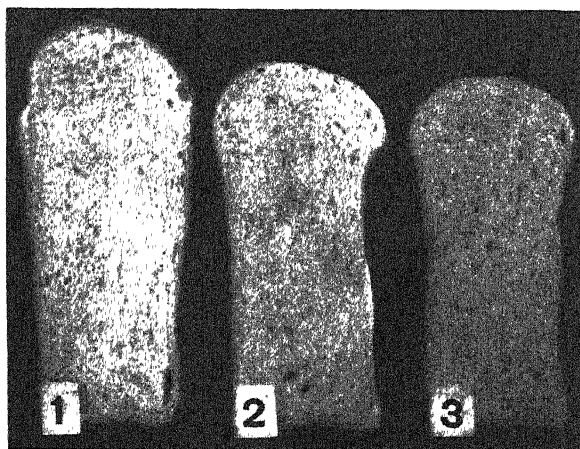


Fig. 3. Loaves made from compressed yeast. Loaf No. 1 was made from fresh yeast, loaf No. 2 from yeast frozen one month, and loaf No. 3 from yeast frozen 3 months

Bread from another lot of yeast frozen three months fermented about 60 minutes longer, while that from two lots frozen 11 to 12 months required around $2\frac{1}{2}$ hours of additional time. Yeast from a third lot frozen 12 months did not lighten the dough sufficiently for baking. The doughs were fermented in calibrated expansion tubes and pan

proofed in uniform test pans. Loaves from fresh compressed yeast and from yeast frozen for one and three months are shown in Figure 3.

It is evident from Figure 3 that the quality of bread made from the frozen yeast was good, as is also indicated by the judges' scores in Table IV. A lowering of quality was noted in the bread made from yeast frozen for long periods. Although these loaves required longer fermentation to reach the same degree of lightness, they were still fair in quality, as can be noted from their ratings and from the loaves shown in Figure 4.

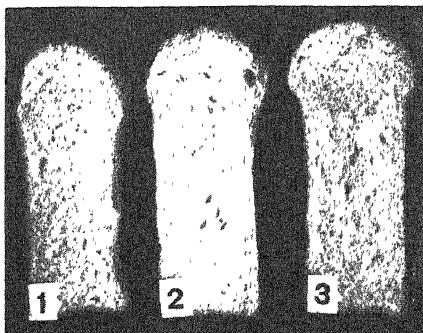


Fig. 4. Loaves made from frozen compressed yeast. Loaf No. 1 was made with yeast frozen 4 months, loaf No. 2 with yeast frozen 11 months, and loaf No. 3 from yeast frozen 12 months.

Bailey (1940) reported somewhat greater deterioration in frozen yeast stored at 20°F than was evident in the Wyoming tests, which may have been due to differences in the viability of the yeast or to methods of storage and handling. In Bailey's tests, pound cakes were frozen but were not in special wrappings or containers. A part of the yeast was thawed over night before being mixed into a dough. In the tests reported here, small cakes were left wrapped in foil, and after opening, were rewrapped in waxed paper for continued storage. A part was stored in a practically air-tight and moisture-proof metal container in the freezing unit, and the remainder was left in the freezing tray. The frozen yeast was used immediately when removed. It was thawed in lukewarm water (83°F) before being mixed into a dough.

The extent to which the yeast cells survived the low temperatures when stored for varied time periods was further measured by staining with methylene blue, which stains the dead but not the living cells. The following technique was used: Suspensions were made from the fresh and also from varied lots of the stored and frozen compressed yeast by adding a weighed amount to a phosphate buffer according to

the recommendations of Fink and Kuhle (1933) and Mills (1941).¹ A buffer with a pH of 4.6 seemed most favorable for preventing an increase in the number of stained cells during the counting. Such an increase was evident with a pH value of 7 and above.

Methylene blue was added in an amount to make the dye concentration 1 : 10,000 as recommended by Mills (1941).¹ The staining was preferred to plate counts as it was much speedier. Moreover, plate counts, in Mills' opinion, gave the number of reproducing cells rather than a representative count of the viable cells in the fermenting culture.

One drop of each suspension was examined under the microscope and counts were made on a Thoma-Zeiss haemocytometer. The counts on each lot were made on about 100 small square areas or until the total averaged around 1200 cells. The formula for calculating the percent of dead cells by staining was

$$\frac{S}{U + S} \times 100$$

where S represents the number of stained cells and U the number of unstained cells.

The time interval had little effect on numbers of stained cells in the buffered solution, whereas in distilled water there was an increase of 28% after several hours.

TABLE IV

THE RELATIONSHIP OF THE NUMBER OF DEAD CELLS IN FROZEN YEAST TO PRESSUREMETER READINGS, FERMENTATION AND SCORING OF LOAVES

Sample	Condition	Age yeast	Dead cells	Pressure meter reading	Time required for dough fermentation and proofing	Baking value, basis of 100
			%	mm Hg		
1	Not frozen	Fresh	1.7	505	4 hr 30 min	95
2	Frozen	1 mo	6.0	495	4 hr 50 min	90
3	Frozen	3 mo	9.6	490	5 hr 33 min	90
4	Frozen	4 mo	11.03	450	5 hr 45 min	90
5	Frozen	11 mo	21.9	340	6 hr 50 min	85
6	Frozen	12 mo	25.0	320	7 hr	70

The loss of living cells, the lessening of the activity of the yeast in a dough, and the rating of the bread made from yeast frozen for varied times are given in Table IV.

A gradual loss in viability of the frozen yeast during storage is evident in Table IV. The data show that the yeast held up surprisingly well for several months. A loss of 10% to 14% of the cells

¹ Note Appendix.

seemingly did not slow up fermentation to the extent that the yeast was undesirable for bread. The longer proofing time up to a certain point, with a high-grade flour, did not seem to affect adversely either the flavor or quality of the loaf, as is evidenced by the scores in Table IV. The ratings were made by trained judges on unidentified loaves. The baking values indicate that bread of good quality was made from yeast frozen for periods up to four months.

Yeast from different lots showed considerable variability. Some lots withstood the long storage period at low temperatures better than others. Counts revealed that from 1.7% to 2.4% of the cells were stained in fresh cakes from several different lots purchased from the grocer and tested at once upon delivery, whereas 6.2% to 8.5% were stained in different lots frozen for two months. In one lot frozen a year 22% of the cells were dead, whereas in others frozen a similar time, 31% to 50% were stained.

The appearance and physical condition of yeast frozen over a period of time and stored in containers was much better than that kept in an open tray. A portion of the latter was dark, dry, and covered with mold. For the most part the lots of frozen yeast, if well wrapped, were in an excellent physical condition and retained their natural color. This yeast frequently became sticky and difficult to handle when thawed. One satisfactory method for handling was thawing in lukewarm water at 83°F. A second method, in which the yeast was allowed to thaw at room temperature over night before incorporation into a dough, proved less satisfactory. This was no doubt due to loss in viability after removal to the warm temperature, as shown in Table V.

TABLE V
DETERIORATION OF FROZEN YEAST AFTER THAWING

Cake	Months frozen	Length of time left at room temperature upon removal from storage	Dead cells %
1	4 mo	Tested at once	11.03
1	4 mo	Four hours, unwrapped	12.8
1	4 mo	Twelve hours, unwrapped	33.8
1	4 mo	Eighteen hours, unwrapped	70.03

It is of interest to note that living organisms such as yeast can withstand freezing temperatures over a period of time and resume almost normal biological activity when placed under favorable environmental conditions, such as the incorporation of the yeast into a sponge or dough, with fermentation at 83° to 85°F. It is well known that high temperatures have the opposite effect.

It has been evident from this study that both dry and moist yeast may be stored at low temperatures over a period of months and still produce good bread. The viability of the stored yeast may be speedily measured from time to time by using the methods described.

Summary

Both dry and compressed yeast were active over much longer periods when stored at temperatures of 40° to 50°F than when stored at room temperatures of 70° to 75°F. The activity of the yeast was measured by fermenting in a Sandstedt-Blish pressuremeter and by bread baking tests. The pressuremeter method proved a speedy and excellent way to test the viability of both dry and moist yeasts.

Compressed yeast was well preserved for several months by freezing and resumed almost normal biological activity when mixed into a dough and placed under favorable environmental conditions.

Staining with methylene blue proved a speedy method for determining the viable cells in stored moist yeast. A phosphate buffer was added in order to prevent an increase in the number of stained cells during the counting.

Microscopic examination revealed a comparatively small percentage of dead cells in moist yeast frozen one to three months. The percentage increased with longer storage. A loss of 10% to 14% of the cells did not slow up dough fermentation to the extent that the yeast was unsuitable for bread.

Acknowledgments

The author is indebted to the Northwestern Yeast Company and Standard Brands, Inc., for yeast to carry on these tests and to Laurens Anderson, technical assistant, for preparation of the buffer and stain.

Literature Cited

- Bailey, L. H., Bartram, M. T., and Rowe, S. C.
1940 Effect of storage temperatures upon the viability and baking properties of compressed yeast. *Cereal Chem.* 17: 55-66.
- Bohn, R. T., and FAVOR, H. H.
1939 Evaluation of yeast activity by means of the Sandstedt-Blish pressure meter. *Cereal Chem.* 16: 238-252.
- Doty, J. M., and Urban, W. R.
1940 The pressuremeter in the study of yeast. *Cereal Chem.* 17: 44-54.
- Fink, H., and Kuhles, R.
1933 Staining yeast with methylene blue. *Wochschr. Brau.* 50: 185. (Abstract quoted.)
- Iwanowski, W., and Brezezinski, K.
1934 Changes in pressed yeast during storage. *Przemysl Chem.* 18: 93-101. (Abstract quoted.)
- Mills, Dale
1941 Differential staining of living and dead yeast cells. *Food Research* 6: 361-371.
- Weaver, R., Talbott, P., and Coleman, D. A.
1933 Variability in experimental baking. II. Yeast variability. *Cereal Chem.* 10: 617-618.

APPENDIX

Buffer and Stain for Yeast Cell Counts

LAURENS ANDERSON, TECHNICAL ASSISTANT

A. The buffer was made 0.2M in NaH_2PO_4 , the concentration suggested by Mills (1941). The concentration of Na_2HPO_4 required to give a pH of 4.6 was determined by experiment, and was found to be 0.004M. At the same time the value of pK in the physico-chemical equation

$$\text{pH} = pK + \log \frac{B}{A} \quad (1)$$

was computed, as the value of 6.8 given in the literature for the phosphate buffer system does not fit at these concentrations. In the above equation, B represents concentration of Na_2HPO_4 and A represents concentration of NaH_2PO_4 , expressed in moles per liter.

Putting the experimentally determined values of pH and B into equation 1, with $A = 0.2$, and solving for pK we get 6.3 for this constant.

A suggested procedure for making this phosphate buffer is as follows (quantities for 650 ml buffer):

1. Make a saturated NaH_2PO_4 solution as follows: Dissolve 70 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 43 ml water by heating in a boiling-water bath. Filter the hot solution and place in constant-temperature bath at 25°C until crystallization begins, and let stand 12 hours after this. (Total time will be at least 24 hours.) Decant the solution from the crystals, and pipette 25 ml of the solution, free from crystals, into a 1,000-ml graduate.

2. Make a saturated Na_2HPO_4 solution in the same manner, dissolving 40 g $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ in 49 ml H_2O . Decant after crystallization, taking care that no crystals are in contact with the solution which is to be used.

3. Dilute the 25 ml of sat NaH_2PO_4 sol to 726 ml with water. Remove 80 ml of this solution.

4. Measure 35.36 ml of the saturated Na_2HPO_4 from a burette into a 50-ml volumetric flask, and dilute to the mark. This gives 0.6M Na_2HPO_4 .

5. Add 4.33 ml of the 0.6M Na_2HPO_4 from a microburette to the 646 ml of dilute NaH_2PO_4 in the graduate.

6. Determine the pH of the resultant mixture. If the steps in the procedure so far have been carried out carefully, the buffer should have a pH of 4.6, and should have the composition: $\text{NaH}_2\text{PO}_4 = 0.2M$, and $\text{Na}_2\text{HPO}_4 = 0.004M$.

7. (a) If the pH is less than 4.6, add more Na_2HPO_4 solution, calculated as follows:

Let the amount to be added, measured in ml, = C , then

$$C = \frac{\text{volume of buffer in graduate} \times (0.004 - B)}{0.6}$$

where B is calculated from the equation:

$$B = \frac{1}{\text{antilog}(7.0 - \text{pH})}$$

Use the value of pH as determined above (6) for this calculation. The calculation is based on the assumption that the concentration of Na_2HPO_4 is less than 0.004M. While the assumption is not necessarily true, calculations based on it will give amounts of the phosphate solution to be added to give a pH of 4.6. Solving the equation $\text{pH} = pK + \log B/A$:

$$\log B - \log A = \text{pH} - pK$$

$$\log B = \text{pH} - pK + \log A$$

Changing signs, and substituting known values for pK and A ,

$$\log \frac{1}{B} = 6.3 - \text{pH} - \log 0.2$$

$$\log \frac{1}{B} = 6.3 - (-0.7) - \text{pH} = 7.0 - \text{pH}$$

$$\frac{1}{B} = \text{antilog}(7.0 - \text{pH})$$

$$B = \frac{1}{\text{antilog}(7.0 - \text{pH})} = \text{actual concentration of } \text{Na}_2\text{HPO}_4 \text{ now in the buffer.}$$

Now the amount of the Na_2HPO_4 solution to be added, designated by C , is equal to the number of millimoles needed, divided by 0.6, since 0.6 is the molarity of the stock Na_2HPO_4 ; and the number of millimoles needed is equal to the volume of buffer in the graduate $\times 0.004$, minus this volume $\times B$, its molarity. Or,

$$C = \frac{\text{volume of buffer in graduate} \times (0.004 - B)}{0.6}$$

(b) If the pH is over 4.6, add an amount of the NaH_2PO_4 solution saved from step described above (3), calculated as follows (D = amount to be added):

$$D = \frac{\text{volume of buffer in graduate} \times (B - 0.004)}{0.004}$$

where B is calculated as above.

This calculation is based on the assumption that the concentration of Na_2HPO_4 is more than 0.004M. The volume to which the buffer must be diluted with NaH_2PO_4 solution is equal to the number of millimoles of Na_2HPO_4 present divided by 0.004. And the number of millimoles of Na_2HPO_4 present is equal to the volume of buffer in the graduate $\times B$. Further, the amount of NaH_2PO_4 solution to be added, designated by D , is equal to the final volume minus the volume already in the graduate. This gives:

$$D = \frac{\text{volume of buffer in graduate} \times (B - 0.004)}{0.004}$$

8. Redetermine the pH, and if necessary, repeat step 7

B. Preparation of methylene blue stain for a 1 : 10,000 concentration: A dropper was calibrated and found to drop 22 drops of 0.037M methylene blue solution per milliliter. The yeast solution to be counted was made by adding 0.10 g of yeast to 40 ml of buffer. The amount of dye needed to make the concentration 1 : 10,000 was calculated as follows:

Wt 40 ml water at 25°C.	39.9 g
Wt yeast	0.1 g
Wt 7 drops dye.	0.3 g
Total wt of suspension	40.3 g

$\frac{40.3}{10,000} = 0.00403$ g = amount of dye needed in yeast suspension. Therefore the dye solution must contain $\frac{22}{7} \times 0.00403 = 0.01267$ g methylene blue per ml.

$\frac{0.5}{0.01267} = 39.46$ = volume of dye solution containing 0.5 g methylene blue. Therefore, 0.5 g of methylene blue in 39.5 ml solution is equal to 1.27 g per 100 ml or 0.0127 g per ml. The molarity of this dye solution is then

$$\frac{0.0127 \times 1000}{\text{molecular wt methylene blue}} = \frac{12.7}{373.73} = 0.034M.$$

YEAST FERMENTATION AND POTASSIUM BROMATE AS FACTORS INFLUENCING THE HARMFUL EFFECTS OF WHEAT GERM ON BAKING QUALITY¹

DONALD E. SMITH and W. F. GEDDES

Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minnesota

(Read at the Annual Meeting, May 1941)

It is well known that wheat germ has a harmful effect upon the baking characteristics of wheat flour, as indicated by the inferior handling properties of the dough and the production of small loaves possessing "green" or underfermented characteristics. Extension of the fermentation time, heat treatment, or water extraction of the germ and the addition of such oxidizing agents as potassium bromate to the dough decrease the deleterious effects. The literature on this subject has recently been reviewed and discussed by Sullivan, Howe, Schmalz, and Astleford (1940) and by Shen and Geddes (1942) and need not be detailed here.

It is sufficient to state that since the early studies reported by Geddes (1930) on the influence of wheat germ on baking quality, it has been established that a water-soluble reducing substance—glutathione—is the main factor responsible for its injurious baking effects. Moreover, it has been discovered that the flour proteinases, like papain, are inhibited by the common oxidizing agents which act as flour improvers, while they are activated by certain reducing agents such as sulfhydryl compounds and hydrogen sulfide. Some investigators believe that the improving action of oxidizing agents is due to their inhibitory effect on the flour proteases, while others hold that the reducing substances present in flour exert a direct action on the gluten proteins, since the effect of added glutathione on the physical properties of bread doughs is too rapid to be attributed solely to enzyme action. Moreover, the improvement noted in dough properties and bread characteristics by lengthening the fermentation time in the baking of germ-flour mixtures is incompatible with the theory that proteinase activation is primarily responsible for the harmful effects of wheat germ.

Recently Shen and Geddes (1942) determined the amino nitrogen and reducing matter contents of nonbromated and bromated doughs made from patent, fancy clear, and low-grade flours after varying fermentation times. These determinations were also carried out on similar doughs in which yeast activity was inhibited by the addition

¹ Paper No. 1,990, Scientific Journal Series, Minnesota Agricultural Experiment Station. Condensed from a thesis presented by Donald E. Smith to the faculty of the graduate school in partial fulfillment of the requirements for the degree of Master of Science, March, 1942.

of octyl alcohol and an attempt was made to correlate these data with the baking behavior of the flours. With an increase in proteolytic activity and reducing matter content of the flours, a longer fermentation time and/or a higher bromate dosage was required to secure optimum dough handling properties and loaf characteristics. These supplementary baking effects of fermentation and bromate treatment could not readily be explained by the results of the amino nitrogen and reducing-matter determinations on either the fermenting or nonfermenting doughs. Thus, with few exceptions, the reducing-matter content of the basic doughs increased with fermentation, whereas it was decreased by the addition of bromate. Moreover, the amino nitrogen content of the nonfermenting doughs increased with increasing rest time. (In the fermenting doughs the amino nitrogen decreased with time of fermentation as a result of its utilization by the active yeast cells and hence could not be used as a measure of increased proteolysis with time.) The amino nitrogen contents of the bromated doughs were, however, lower than those of the corresponding nonbromated doughs, particularly in the presence of active yeast fermentation.

The supplementary baking effects of fermentation and bromate treatment noted by Geddes (1930) with germ-flour mixtures and by Shen and Geddes (1942), particularly with the fancy clear and low-grade flours, naturally suggested that the harmful effects of germ might be significantly reduced by fermenting it with yeast in the presence of oxidizing agents before mixing with the flour and other dough ingredients. The experiments reported here were accordingly undertaken to investigate the effects of various pretreatments of wheat germ on its baking properties and to follow the changes in amino nitrogen and reducing-matter content resulting from these treatments. It appeared that such a study might throw further light on the relative importance of proteolytic activity and reducing matter content in relation to flour improvement by oxidizing agents.

During the course of these experiments Hullett (1940) published a brief note describing a method for treating wheat germ so that when it is incorporated with the flour the adverse effects normally associated with its presence are largely avoided. This method involves pre-fermenting the germ for a sufficient length of time to oxidize the glutathione, as indicated by failure to give the nitroprusside reaction. The germ sponge is then mixed with the other dough ingredients and baked in the usual manner. With the use of 4% raw wheat germ (on a flour basis) the bread made by this method was very similar to ordinary white bread; with 10% germ, the volume and texture were only slightly affected but the crumb was light brown in color. From

these observations a commercial process was devised which made it possible to produce an acceptable germ loaf containing up to 10% of wheat germ on a flour basis.

Hullett also noted that improved baking results were obtained by prefermenting the low-grade portions of long-extraction flours and suggested that glutathione elimination by yeast may play a significant part in the ordinary dough ripening process. In a later paper published after the completion of the present study, Hullett and Stern (1941) reported the experiments carried out in developing the commercial process. The elimination of reduced glutathione, as indicated by a negative nitroprusside test, was associated with the fermenting activity of the yeast, since iodoacetic acid, sodium fluoride, and acetone, which inhibited fermentation, also prevented glutathione destruction. The destruction of glutathione apparently is connected with an enzyme mechanism effective in the raw germ, since destruction did not take place when boiled germ was fermented; moreover, destruction took place when glutathione was added to unboiled germ ferments, but not when it was added to a boiled germ ferment or to a fermenting sugar solution. These workers report that the disappearance of the glutathione reaction as a result of fermentation cannot be ascribed to oxidation to the S-S form since treatment with various reducing agents failed to restore the nitroprusside test. In view of these observations they express doubt that the action of potassium bromate in a bread dough is actually an oxidizing one. It was also observed that germ fermentation not only eliminated the glutathione in the germ but also in the yeast and that a brown to pink color began to develop on the surface of the ferment as soon as a negative nitroprusside reaction was obtained.

Experimental

Materials and methods: The experimental materials consisted of a highly refined untreated second middlings flour (12.5% protein, 0.36% ash, 13.5% moisture basis) and commercial wheat germ (27.3% crude protein, 9.9% petroleum ether extract, 13.5% moisture basis), freshly milled from hard red spring wheat. The germ, which contained 10.4% moisture, was ground in a Wiley laboratory mill to pass the 0.5-mm sieve and stored, along with the flour, at approximately 3°C when not in use.

In the major series of experiments, baking tests were conducted by the A. A. C. C. basic method (as outlined in *Cereal Laboratory Methods*, 4th ed., 1941) and also by a bromate method (basic formula +0.001% KBrO_3) on the middlings flour alone and with 5% and 10% of the flour replaced by wheat germ, using dough fermentation times

of 1.5, 3.0, and 4.5 hours. The germ used in these tests was submitted to the following treatments: (1) control—germ added directly to the flour, and (2) pretreated by allowing to stand or ferment in aqueous suspension for 1.5, 3.0, 4.5, 6.0, and 16 hours, respectively, with the following additions: nil, potassium bromate, yeast, and yeast plus potassium bromate. The control series represents the regular basic and bromate straight-dough experimental baking test, while the germ pretreatments involve variations of a sponge and dough baking method in which part of the water, plus the potassium bromate and/or the yeast as specified above, is included in the sponge.

In making up the germ sponges 2 ml of water per gram of germ was used, since it yielded a mixture from which the germ did not settle out. At the end of the sponge time the remaining liquid and the regular ingredients not added to the sponge were mixed in the usual manner to form the dough. In all cases where wheat germ was present it replaced an equivalent dry-matter weight of flour. Three hundred grams of flour or flour and germ and proportional quantities of the other ingredients were mixed in a Hobart-Swanson mixer for two minutes and three 150-g portions scaled off, one for each of the three fermentation times. The punching and molding schedules for the various dough fermentations and other details of the baking method were the same as those described by Shen and Geddes (1942).

In the experiments outlined above only nil and 1 mg KBrO_3 per 100 g of germ and flour were used. A similar series of experiments was carried out with a germ pretreatment time of 3.0 hours and bromate levels of 0, 1, 2, 4, and 6 mg of KBrO_3 with 5% germ and 0, 2, 4, 6 and 10 mg of KBrO_3 with 10% germ.

Baking tests were also conducted in which 10%, 15%, and 20% of the flour was replaced by wheat starch, untreated and pretreated germ, respectively. The wheat starch was prepared in the laboratory from hard red spring wheat flour. The "pretreated" germ required for each loaf was fermented for three hours with 3 g of yeast and 1 mg of KBrO_3 .

In another experiment, wheat germ was continuously extracted with petroleum ether for 24 hours and the extracted germ, after allowing the solvent to evaporate, employed in baking tests in comparison with unextracted germ. In these tests the original and extracted germ samples were used in 5%, 10%, and 15% levels and in each case the germ was employed in the straight-dough baking method and also in a sponge and dough procedure in which the germ required per loaf was prefermented 3 hours in a sponge with 3 g of yeast.

It must be emphasized that the sponge treatments involving yeast and potassium bromate are not directly comparable for differing germ

TABLE I
MEAN LOAF VOLUME DATA FOR PATENT FLOUR AND 5% GERM-FLOUR MIXTURES
CONTAINING GERM SUBJECTED TO VARIOUS TREATMENTS

Dough temperature	Patent flour	Loaf-volume data for germ-flour mixtures, with germ pretreated for various periods					
		0 hr	1.5 hrs	3 hrs	4.5 hrs	6 hrs	16 hrs
	Basic	GERM SUSPENSION					
1.5	710	540	530	555	530	530	570
3.0	650	555	520	600	570	550	640
4.5	600	560	555	565	620	570	610
	1 mg BrO ₃	GERM SUSPENSION + 1 MG BROMATE					
1.5	705	565	520	560	575	540	615
3.0	690	615	570	560	580	565	685
4.5	615	650	540	580	570	545	690
	1 mg BrO ₃	GERM SUSPENSION (1 MG BROMATE IN DOUGH)					
1.5	705	565	525	550	555	560	635
3.0	690	615	610	640	655	625	685
4.5	615	650	640	645	660	630	635
	Basic	GERM SUSPENSION + YEAST					
1.5	710	540	560	675	670	730	705
3.0	650	555	600	665	690	710	710
4.5	600	560	585	620	625	680	580
	1 mg BrO ₃	GERM SUSPENSION + 1 MG BROMATE + YEAST					
1.5	705	565	630	670	710	705	695
3.0	690	615	655	690	690	685	650
4.5	615	650	630	645	640	645	630
	1 mg BrO ₃	GERM SUSPENSION + YEAST (1 MG BROMATE IN DOUGH)					
1.5	705	565	605	680	705	705	—
3.0	690	615	705	675	660	655	—
4.5	615	650	620	655	580	535	—

percentages incorporated with the flour at the dough stage. In order to obtain a similar yeast and bromate concentration in the dough, a constant quantity of each had to be used in the sponge; thus with 5% germ-flour loaves, the ratio of germ to yeast in the pretreatment was 5 : 3, whereas with the 10% germ-flour loaves the ratio was 10 : 3.

TABLE II

MEAN LOAF VOLUME DATA FOR PATENT FLOUR AND 10% GERM-FLOUR MIXTURES CONTAINING GERM SUBJECTED TO VARIOUS TREATMENTS

Dough fermenta- tion	Patent flour	Loaf-volume data for germ-flour mixtures, with germ pretreated for various periods					
		0 hr	1.5 hrs	3 hrs	4.5 hrs	6 hrs	16 hrs
hrs	cc	cc	cc	cc	cc	cc	cc
	Basic	GERM SUSPENSION					
1.5	710	500	490	525	525	485	570
3.0	650	490	500	510	500	490	580
4.5	600	520	500	510	500	485	485
	1 mg BrO ₃	GERM SUSPENSION + 1 MG BROMATE					
1.5	705	520	470	515	530	510	535
3.0	690	555	530	525	515	510	570
4.5	615	580	515	525	510	505	465
	1 mg BrO ₃	GERM SUSPENSION (1 MG BROMATE IN DOUGH)					
1.5	705	520	495	520	530	530	570
3.0	690	555	515	530	540	535	610
4.5	615	580	520	525	545	510	500
	Basic	GERM SUSPENSION + YEAST					
1.5	710	500	520	575	570	600	600
3.0	650	490	520	565	535	560	585
4.5	600	520	480	490	480	500	435
	1 mg BrO ₃	GERM SUSPENSION + 1 MG BROMATE + YEAST					
1.5	705	520	535	585	610	595	600
3.0	690	555	545	590	575	560	565
4.5	615	580	485	520	510	490	390
	1 mg BrO ₃	GERM SUSPENSION + YEAST (1 MG BROMATE IN DOUGH)					
1.5	705	520	535	605	595	615	590
3.0	690	555	565	570	550	575	590
4.5	615	580	490	490	510	505	455

Amino nitrogen and reducing matter content were determined upon extracts of treated and untreated wheat germ, employing the methods described by Shen and Geddes (1942). Portions of each of the extracts were tested for sulfhydryl groups by the nitroprusside test.

Determinations of the pH of some of the germ suspensions were made employing a glass electrode.

Baking results: The mean loaf volume data for the patent flours and the 5% and 10% germ-flour mixtures involving various germ treatments are recorded in Tables I to IV; the loaf volumes for the 5% germ treatments are also diagrammatically represented in Figures 1 and 2. While the handling properties of the doughs were noted at various stages of the fermentation and the loaves judged for external appearance, crumb grain, texture, and crumb color, these data have not been tabulated here since they are closely related to loaf volume. Some idea of the general loaf characteristics for certain of the germ treatments may be gained from the photographs reproduced in Figures 3 and 4.

TABLE III

MEAN LOAF VOLUME DATA FOR PATENT FLOUR AND 5% GERM-FLOUR MIXTURES SHOWING EFFECTS OF PREFERMENTATION FOR THREE HOURS AND VARIOUS BROMATE DOSAGES

Dough fermentation hrs	Loaf volume for patent flour		Loaf volumes for germ-flour mixtures with various dosages of KBrO_3 in mg. ¹				
	Basic	1 mg KBrO_3	0	1	2	4	6
cc	cc	cc	cc	cc	cc	cc	cc
			GERM SUSPENSION WITH BROMATE				
1.5	710	705	560	560	565	620	645
3.0	650	695	600	565	595	725	710
4.5	600	615	565	580	575	675	645
			GERM SUSPENSION (BROMATE IN DOUGH)				
1.5	710	705	560	550	630	700	740
3.0	650	695	600	640	715	920	660
4.5	600	615	565	665	670	520	450
			GERM SUSPENSION + YEAST WITH BROMATE				
1.5	710	705	675	675	740	690	—
3.0	650	695	665	690	725	620	—
4.5	600	615	620	650	665	570	—
			GERM SUSPENSION + YEAST (BROMATE IN DOUGH)				
1.5	710	705	675	680	740	770	—
3.0	650	695	665	675	655	590	—
4.5	600	615	620	655	565	455	—

¹ The germ pretreatment consisted of standing in aqueous suspension for three hours with and without yeast and/or potassium bromate as indicated.

TABLE IV
MEAN LOAF VOLUME DATA FOR PATENT FLOUR AND 10% GERM-FLOUR MIXTURES
SHOWING EFFECTS OF PREFERMENTATION FOR THREE HOURS
AND VARIOUS BROMATE DOSAGES

Dough fermentation	Loaf volume for patent flour		Loaf volumes for germ-flour mixtures with various dosages of KBrO_3 in mg ¹				
	Basic	1 mg KBrO_3	0	2	4	6	10
hrs	cc	cc	cc	cc	cc	cc	cc
GERM SUSPENSION WITH BROMATE							
1.5	710	705	525	520	530	520	535
3.0	650	695	525	545	560	545	590
4.5	600	615	510	510	535	530	565
GERM SUSPENSION (BROMATE IN DOUGH)							
1.5	710	705	525	520	610	625 ²	610 ²
3.0	650	605	525	560	625	500	515
4.5	600	615	510	550	595	455	410
GERM SUSPENSION + YEAST WITH BROMATE							
1.5	710	705	575	585	—	610 ²	—
3.0	650	695	565	590	—	550	—
4.5	600	615	490	520	—	440	—
GERM SUSPENSION + YEAST (BROMATE IN DOUGH)							
1.5	630 ²	670 ²	575	605	700 ²	675 ²	670 ²
3.0	675	700	565	570	510	495	420
4.5	575	665	490	490	380	370	350

¹ The germ pretreatment consisted of standing in aqueous suspension for three hours with and without yeast and/or potassium bromate as indicated.

² Baking tests in this column conducted with another sample of patent flour obtained from the same source.

When the germ was added directly to the flour and baked by the basic formula, the doughs were soft and sticky upon removal from the mixer, particularly those containing 10% of wheat germ. As fermentation progressed, the handling properties improved. The loaves baked from the basic doughs after only 1.5 hours of fermentation possessed pronounced "green" or underfermented characteristics. They were small in volume, had sharp corners, a glossy side crust with small round holes, a reddish top crust, flat top and no break; the cells of the crumb were large and round with thick cell walls. As the fermentation was extended to 4.5 hours, the dough-handling properties improved and the loaves were somewhat larger in volume and had less pronounced underfermented characteristics.

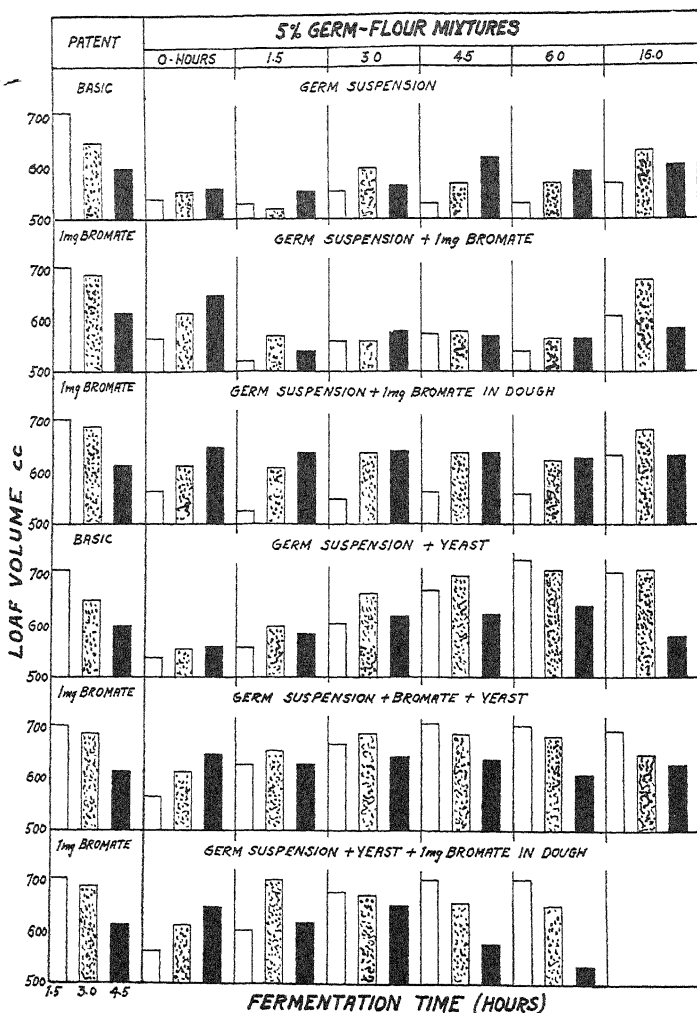


Fig. 1. Effect of wheat germ on the loaf volume of 5% germ-flour mixtures at dough fermentation times of 1.5, 3.0, and 4.5 hours. Diagrams show the effects of adding untreated germ at the dough stage (0 hours) as compared with germ added after various sponge treatments, including standing in aqueous suspension (with and without KBrO_3) and yeast fermentation (with and without KBrO_3) for various times.

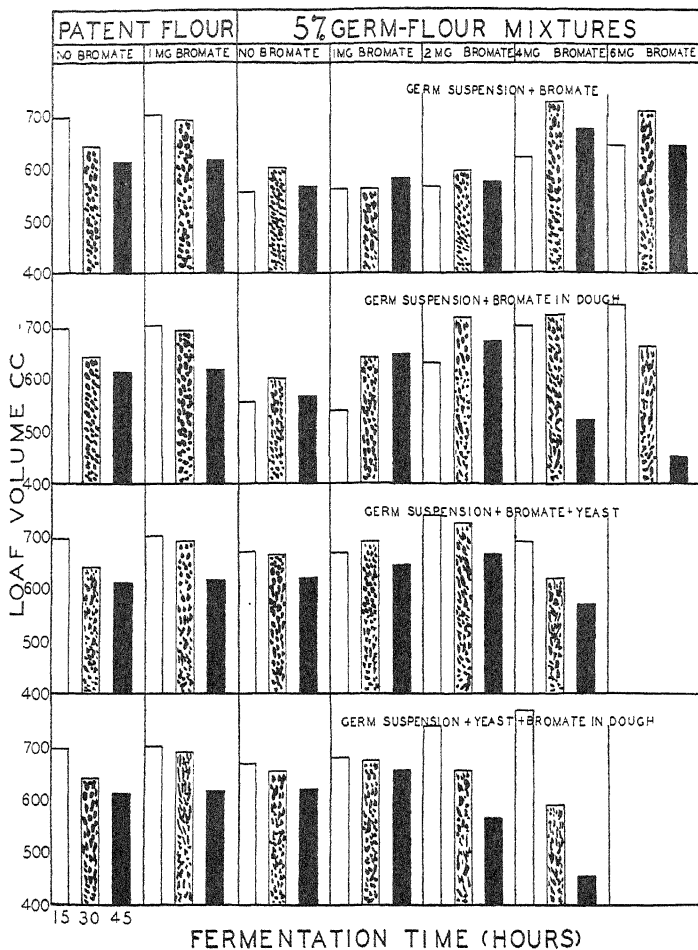


Fig. 2. Effect of wheat germ on the loaf volume of 5% germ-flour mixtures at dough fermentation times of 1.5, 3.0, and 4.5 hours. Diagrams show the effects of adding wheat germ at the dough stage after standing or fermenting for 3 hours with additions of 0, 1, 2, 4 and 6 mg of KBrO_3 per 5 g of germ.

The inclusion of potassium bromate in the baking formula, however, had a very pronounced improving effect when the germ-flour mixtures were baked by the regular straight-dough experimental procedure. The physical properties of the doughs were enhanced shortly after mixing and during the early part of the fermentation but doughs with the highest bromate treatments became "tough" as fermentation












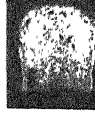




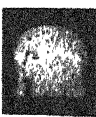
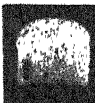
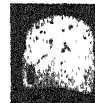
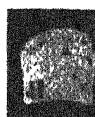




Dough Fermentation Hours	Patent Flour	5% Germ Flour Mixtures		
		Germ Yeast - Prefermentation (hours)		
		0	3	6
1.5				
				
3.0				
				
4.5				
				

Fig. 3. Photographs of loaves baked by basic formula showing the effect of prefermenting germ for various times on the baking properties of 5% germ-flour mixtures.

was extended. This "over effect" was more noticeable with the 10% than with the 5% germ flour doughs. These progressive changes in dough properties were paralleled by changes in the bread character-

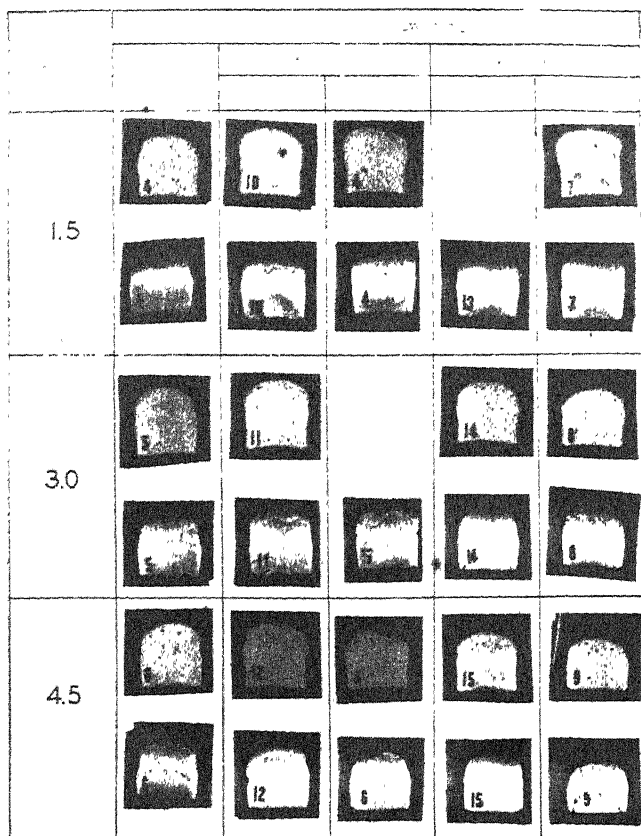


Fig. 4. Photographs of loaves baked from 5% germ-flour mixtures showing the increased efficiency of potassium bromate when added at the dough stage. For all loaves the germ was prefermented 3 hours; those in the left-hand column were baked by the basic formula, while those in the remaining columns were baked with the addition of 2 or 4 mg of KBrO_3 to the germ sponge or dough, as indicated

istics. The underfermented characteristics became less pronounced and loaf volume increased to an optimum and then decreased. As the bromate dosage was increased, the optimum loaf, in general, was obtained at shorter dough fermentation times. The highest bromate dosages of 6 and 10 mg per loaf in the 5% and 10% germ-flour mixtures, respectively, gave loaves possessing slightly "old" characteristics at 4.5 hours of fermentation.

Turning now to the effects of pretreatment, allowing the germ to stand in aqueous suspension for varying periods of time up to and including 6 hours had little influence on its baking effects. A slight

improvement in both the 5% and 10% germ-flour loaves was noted for the 16-hour treatment. In these instances there was evidence of some fermentation, presumably by wild yeasts. The addition of 1 mg of KBrO_3 to the aqueous germ suspension likewise had little effect but, as shown in Tables III and IV and Figures 2 and 4, large dosages (4 and 6 mg in the 5% germ series and 10 mg in the 10% germ series), somewhat decreased the deleterious effects of the germ.

Prefermentation of the germ, however, strikingly reduced its harmful baking effects, the improvement in this regard being supplemented by the presence of potassium bromate in the fermenting germ sponge. The influence of potassium bromate was markedly greater when it was incorporated at the dough stage; in this instance the larger bromate dosages gave doughs that were "short" and "dead" and loaves possessing pronounced overfermented characteristics when the dough fermentation was extended beyond 1.5 hours and especially for the longer germ prefermentation times. These data clearly show that

TABLE V

MEAN LOAF VOLUME DATA BY BASIC FORMULA SHOWING EFFECTS OF CORRESPONDING ADDITIONS OF WHEAT STARCH, UNTREATED GERM, AND PREFERMENTED-BROMATE-TREATED GERM¹

Flour diluent	Loaf volumes with dough fermentation times in hours:		
	1.5 hrs	3.0 hrs	4.5 hrs
	cc	cc	cc
10% DILUENT ADDED			
Wheat starch	625	625	580
Untreated germ	500	490	520
Pretreated germ	585	585	520
15% DILUENT ADDED			
Wheat starch	560	565	570
Untreated germ	470	460	480
Pretreated germ	495	490	400
20% DILUENT ADDED			
Wheat starch	535	535	545
Untreated germ	370	370	380
Pretreated germ	395	335	285

¹ The required quantities of germ per loaf were prefermented 3 hours with 3 grams yeast and 1 mg of KBrO_3 .

the prefermentation of the germ shortens the fermentation required in the dough stage to secure optimum baking results and that potassium bromate is much more effective in reducing the deleterious baking effects of wheat germ when it is added to the dough. It is of interest to note that under optimum conditions the loaf volumes of the germ-flour doughs exceeded that of the patent flour alone; aside from crumb color, the other bread characteristics were eminently satisfactory.

The crumb color of these optimum loaves was greyish-yellow. Loaves representative of undertreatment of the germ possessed a yellowish-brown crumb, while those in which the germ was overtreated exhibited a distinct reddish-brown crumb, the intensity of the reddish cast being more or less proportional to the extent of the overtreatment as indicated by the decrease in loaf volume from the optimum. The surface of the fermenting germ suspensions also developed a brown color with time.

The loaf-volume data recorded in Table V for patent flour diluted with 10%, 15%, and 20% wheat starch, untreated wheat germ, and

TABLE VI
MEAN LOAF VOLUME DATA FOR 5%, 10%, AND 15% GERM-FLOUR MIXTURES
SHOWING EFFECTS OF PREFERMENTATION ON UNTREATED AND
PETROLEUM-ETHER-EXTRACTED GERM

Dough fermentation time	Loaf volume					
	Unextracted germ, germ level in %:			Petroleum-ether-extracted germ, germ level in %:		
	5%	10%	15%	5%	10%	15%
hrs	cc	cc	cc	cc	cc	cc
GERM ADDED DIRECT TO DOUGH						
1.5	540	500	470	550	530	—
3.0	555	490	460	580	520	—
4.5	560	525	480	565	490	—
GERM PREFERMENTED 3.0 HOURS						
1.5	675	575	495	680	565	410
3.0	640	565	490	665	535	365
4.5	620	490	400	625	440	315

fermented wheat germ (3.0 hours with 1 mg KBrO_3 present), respectively, show quite clearly that the harmful baking effects of the germ are not due merely to a dilution effect. Moreover, these data indicate that prefermentation of the germ is relatively ineffective with germ levels exceeding 10%. That this decrease in the relative efficiency of prefermentation with large germ concentrations is not due primarily to the presence of larger amounts of lipids in the dough, which *per se* have a harmful effect on gluten, is shown by the comparative loaf-volume data for 5%, 10%, and 15% germ-flour loaves (Table VI) containing unextracted germ and petroleum-ether-extracted germ, with and without prefermentation. The responses of the 5% germ-flour loaves made from unextracted and extracted germ to prefer-

mentation, respectively, are virtually identical while the 10% and 15% germ-flour series show larger volumes and greater responses to pre-fermentation in the instance of the unextracted germ.

Physico-chemical and chemical tests: In our hands the nitroprusside reaction did not prove to be particularly sensitive. Patent flour extracts gave a negative test and untreated germ extracts gave definitely positive tests; on the other hand extracts of 5% germ-flour mixture gave an indecisive test. When wheat germ stood in aqueous suspension with or without the addition of potassium bromate strong positive tests were obtained; pre-fermentation, however, progressively decreased the intensity of the color produced, but it was impossible to determine with any degree of certainty the fermentation time required to secure a negative test or whether the inclusion of potassium bromate in the fermenting germ sponge decreased this time. It must be pointed out, however, that no particular effort was directed to perfecting the technique of this qualitative test since quantitative determinations of reducing matter content were also being carried out.

The results of pH determinations on nonfermenting and fermenting germ suspensions, with and without the addition of potassium bromate, are recorded in Table VII. Wheat germ has a relatively high

TABLE VII
EFFECT OF FERMENTATION AND POTASSIUM BROMATE ON pH OF WHEAT
GERM SUSPENSIONS

Nature of suspension	Time of standing in hours.		
	0 hrs	1.5 hrs	3.0 hrs
Aqueous germ suspension	pH 6.4	pH 6.4	pH 6.6
Aqueous germ suspension + 2 mg KBrO ₃ per 5 g	6.6	6.5	6.5
Aqueous germ suspension + 3 g yeast per 5 g germ	6.1	6.0	6.2
Aqueous germ suspension + 3 g yeast + 2 mg KBrO ₃ per 5 g	6.1	5.9	6.0

buffer capacity and the changes in acidity due to fermentation of the germ appear entirely too small to account for the marked improving effect of pre-fermentation on the baking properties.

Because of lack of experimental material, only a limited number of tests for the effect of germ treatment on proteinase activity and reducing-matter content was carried out. The mean results for extracts prepared from nonfermenting and fermenting germ suspensions with and without bromate after various time intervals are given in Table VIII. Without yeast or bromate the amino nitrogen and reducing-matter content increased with time of standing, the extent of the increase being much greater for the latter. The inclusion of 4

mg of KBrO_3 in the suspension tended to inhibit proteolysis and the increase in reducing-matter content with time.

When fermentation took place, the amino nitrogen compounds were utilized by the yeast, and at three hours of fermentation time the amino nitrogen was lower than in the original germ or in a nonfermenting suspension allowed to stand for the same length of time. Yeast

TABLE VIII
EFFECT OF GERM PRETREATMENT ON AMINO NITROGEN AND REDUCING
MATTER CONTENT

Treatment time hrs	Aqueous suspensions— KBrO_3 in mg			Yeast suspensions— KBrO_3 in mg.	
	0 mg	2 mg	4 mg	0 mg	4 mg
AMINO NITROGEN CONTENT IN MG PER G OF GERM					
0	2.0	2.2	2.0	—	—
1.5	2.1	—	—	1.3	—
3.0	2.2	—	2.0	1.5	1.3
4.5	2.6	—	—	1.6	—
5.0	2.4	—	—	—	—
REDUCING MATTER CONTENT ML 0.01 <i>N</i> IODINE PER G GERM ¹					
0	1.3	1.3	1.2	—	—
1.5	1.9	—	—	1.6	—
3.0	2.6	—	1.9	1.5	1.0
4.5	2.5	—	—	1.5	—
5.0	2.9	—	—	—	—

¹ Positive nitroprusside tests were obtained with the aqueous germ suspensions, the intensity of the test being reduced when potassium bromate was present. The yeast-germ suspensions gave either negative or doubtful tests at times corresponding to those at which reducing matter content was determined.

fermentation, moreover, greatly inhibited the increase in reducing-matter content, which was observed with time of standing in the instance of the nonfermenting suspensions; the inclusion of 4 mg of KBrO_3 in the fermenting suspension further lowered the reducing-matter content. However, both fermentation and the presence of potassium bromate were required to keep the reducing-matter content at approximately the original level of a freshly made suspension. Yet the germ suspension, fermented for three hours, gave a negative nitroprusside test, whereas the original germ gave a strong positive test.

Discussion

The baking results demonstrate that prefermentation of wheat germ, as first announced by Hullett (1940) and Hullett and Stern (1942), markedly lowers its deleterious effect upon flour baking quality.

Such pretreatment, however, is not particularly helpful when germ levels of 10% and higher are employed. That this decrease in baking response to pretreatment with high germ levels is apparently not to be ascribed to masking by the harmful effects of the germ lipids is shown by a similar lack of response in the instance of prefermented petroleum-ether-extracted germ when used at correspondingly high levels.

An outstanding feature of the results is the difference in the efficiency of potassium bromate as an improver when added to aqueous germ suspensions, fermenting germ suspensions, and to the fermenting germ-flour doughs, respectively. Baker and Mize (1939) have previously shown that bromate has little effect in the absence of both mechanical action and yeast fermentation. When added to the non-fermenting germ suspensions very large dosages were required to effect an improvement in dough handling properties and in general bread quality. The efficiency was considerably increased by adding yeast to the germ sponge but it was much more effective when added at the dough stage. Long prefermentation of germ coupled with large bromate dosages in the doughs resulted in an "excess bromate effect" as shown by bread possessing pronounced overfermented characteristics, particularly with long dough fermentation times. Moreover the "excess effect" appeared to be more pronounced with bread containing more germ. While it is known that the rate of oxidation by bromate increases with an increase in acidity, the changes in pH observed in these studies seem entirely too small to explain the marked differences observed. A complete theory of the nature of bromate action must obviously provide an explanation of the supplementary effects of yeast fermentation and bromate efficiency.

Hullett and Stern (1942) ascribe the beneficial effects of prefermentation to glutathione "destruction," as indicated by their failure to obtain a positive nitroprusside test when the germ was prefermented for a sufficient length of time to secure maximum improvement. In the present studies, similar observations were made but the qualitative indications were not in accord with the quantitative results for reducing matter content, as determined by the Freilich (1941) procedure. On the other hand the reducing-matter values were not correlated with the baking behavior. Thus the reducing-matter content of aqueous germ suspensions increased with time of standing but this was not accompanied by any significant change in the loaf characteristics of flour-germ admixtures in which the germ component had been allowed to stand in aqueous suspension for varying times. Prefermentation, especially when potassium bromate was present, tended to inhibit the increase, but extensive pretreatment, which had

marked baking effects, was necessary to maintain the reducing matter (and amino nitrogen) content at the level present in the original untreated germ.

These observations naturally raise several questions which cannot be answered on the basis of the present studies. What specific substances are being measured by the iodine titration procedure and which of these are of significance in relation to baking behavior? Will a modification of the conditions of the test or other quantitative techniques give more significant results? Tests conducted on the fermenting germ-flour doughs which were not reported in detail in this paper showed that, as observed by Shen and Geddes (1942) in the instance of doughs made from fancy clear and low-grade flours, the reducing-matter content tended to increase with fermentation, the increase being inhibited by bromate. This suggests that S-S linkages in the proteins are being converted to SH groups and that the oxidation of the latter by bromate may have a profound effect upon gluten properties.

The uncertainties regarding the significance of the reducing-matter values and the limited amount of chemical data render it inadvisable to reach any conclusions as to the relative importance of protease activity and reducing-matter content in relation to the improving effect of fermentation and potassium bromate additions. This is further complicated by the fact that, in the presence of yeast fermentation, amino nitrogen loses its significance as an index of proteolytic activity. However, a few tests on octyl-alcohol-treated doughs left no doubt that the addition of germ to flour markedly increased the proteolytic activity, the extent of the increase being less in the presence of potassium bromate. Since, however, an increase in proteolysis gave improved baking results with untreated germ, the improving effect of potassium bromate cannot be ascribed entirely to protease inhibition; if this were the case, continued improvement without any excess effect should be observed with increasing bromate dosages and at the shorter fermentation times. Moreover, one would expect the greatest improving action of bromate to occur when it is added directly to the germ by decreasing the reduced glutathione content and thereby more or less inactivating the germ proteases before they are added to the flour.

The fact that bromate is a more efficient flour improver when added to the fermenting dough rather than to the fermenting sponge, despite the lower effective bromate concentration, suggests that it exerts a direct action on the gluten proteins.

Summary

Baking tests conducted by the A. A. C. C. basic formula on mixtures of varying percentages of commercial wheat germ with a highly refined hard red spring wheat middlings flour using fermentation times of 1.5, 3.0 and 4.5 hours showed that the germ had a marked deleterious effect upon baking quality. The addition of wheat germ to the flour resulted in soft, sticky doughs and the production of loaves of small volume possessing "green" or underfermented characteristics. Dough-handling qualities and loaf characteristics improved as the fermentation time was extended from 1.5 to 4.5 hours. The inclusion of potassium bromate in the baking formula markedly improved dough handling properties and loaf characteristics.

Allowing the germ to stand in aqueous suspension for periods up to 6 hours prior to mixing with the flour had little or no improving action but when potassium bromate was present in the aqueous suspension in relatively large concentrations (4 mg per 5 g of germ) some reduction in the harmful effects of germ on the baking characteristics of 5% and 10% germ-flour mixtures was noted.

Prefermentation of the germ with yeast progressively decreased the harmful effects with time of prefermentation up to about 4.5 hours with 5% germ-flour doughs. Longer prefermentation times gave little further improvement. The presence of potassium bromate in the fermenting germ suspension further improved the baking behavior of the germ-flour mixtures at short dough fermentation periods. Overeffects, resulting in the production of bread with "old" or overfermented characteristics, were obtained with long germ prefermentation periods and/or long dough fermentation periods, particularly when potassium bromate was added at the dough stage.

Under optimum conditions, bread baked from 5% and 10% germ-flour mixtures approached the quality of that baked from the patent flour alone, with the exception of crumb color. Overtreatment of the germ resulted in bread possessing a brownish red crumb.

The improving action due to germ prefermentation and/or potassium bromate was most pronounced in the 5% germ-flour series. These treatments were relatively ineffective with 15% and 20% germ-flour mixtures. Studies with petroleum-ether-extracted germ showed that failure to obtain improvement with the higher germ levels was not due to the larger quantities of germ lipids present in the doughs.

The efficiency of potassium bromate as a flour improver was greatest when used at the dough stage and least when added to aqueous germ suspensions. In the fermenting germ sponges relatively slight

decreases in pH occurred, which seemed insufficient to explain the greater efficiency of bromate in the presence of yeast fermentation.

In aqueous germ suspensions, amino nitrogen and reducing matter content, particularly the latter, increased with time of standing. The presence of 4 mg of potassium bromate per 5 g of germ in the aqueous suspension tended to inhibit proteolysis and the increase in reducing-matter content with time.

In fermenting germ suspensions, the amino nitrogen compounds were utilized by the yeast. Yeast fermentation, particularly in the presence of potassium bromate, greatly inhibited the increase in reducing-matter content which was observed with time in the instance of nonfermenting suspensions.

Prefermentation for a sufficient time resulted in a negative nitroprusside test, despite quantitative levels of reducing matter equal to or exceeding that of the original germ which gave a strong positive nitroprusside test. The reducing-matter values were not correlated with baking behavior.

The much greater efficiency of potassium bromate when added to fermenting germ-flour dough rather than to fermenting germ sponge, despite a lower effective concentration, suggests that it exerts a direct action on the gluten proteins.

Literature Cited

- Baker, J. C., and Mize, M. D.
1939 The relation of mechanical stirring to sponge doughs. *Cereal Chem.* **16**: 294-295.
- Freilich, J.
1941 A method for the determination of reducing matter in flour. *Cereal Chem.* **18**: 129-137.
- Geddes, W. F.
1930 Chemical and physico-chemical changes in wheat and wheat products induced by elevated temperatures. III. The influence of germ constituents on baking quality and their relation to improvement in flour induced by heat and chemical improvers. *Can. J. Research* **2**: 195-213.
- Hullett, E. W.
1940 Note on the use of yeast to destroy glutathione in wheat germ for bread-making. *New Zealand J. Sci. and Tech.* **22**: 44B-47B.
- Hullett, E. W., and Stern, Rosa
1941 Biological elimination of glutathione from wheat germ and flours used in breadmaking. *Cereal Chem.* **18**: 561-572.
- Shen, Ting, and Geddes, W. F.
1942 Protease activity and reducing matter content of wheat flour doughs in relation to baking behavior. *Cereal Chem.* **19**: 609-631.
- Sullivan, B., Howe, Marjorie, Schmalz, F. D., and Astleford, G. R.
1940 The action of oxidizing and reducing agents in flour. *Cereal Chem.* **17**: 507-528.

THE DETERMINATION OF FLOUR PARTICLE SIZE ¹

F. C. HILDEBRAND, C. G. FERRARI, L. F. BORCHARDT, and C. A. ANKER

General Mills, Inc., Research Laboratories, Minneapolis, Minnesota

(Read at the Annual Meeting, May 1942)

Measurements of particle size have been made on materials ranging from mine-run coal and other minerals to carbon black and rouge for glass polishing. The methods have been diverse, and frequently a method adequate for one type of material has proved totally unsatisfactory for another. In consequence the literature on particle-size measurement is voluminous and in many cases not pertinent to measurements on flour. Brief mention will be made, however, of various types of measurements which have been employed in the past and indications of their applicabilities for flour studies will be given.

Sieving is the method that first suggests itself. In this procedure the weight of material that passes a screen of one size and fails to pass a screen of smaller size is determined. Such measurements provide certain size-distribution data and appear to be simple and direct. However, close study of sieving has revealed serious shortcomings. Aside from the necessity for controlling such features as time of sifting and sieve load, other difficulties are inherent in the method in its application to flour. The tendency of flour to agglomerate seriously interferes with accuracy. Dusting causes errors in the small-size range. Sieves are not accurate beyond a lower limit of approximately 200-mesh. Since a large portion of the total flour weight normally lies below this limit it must be concluded that the procedure is not satisfactory for complete characterization of particle size distribution.

Of the many other methods described in the literature, liquid- and air-elutriation methods do not appear to be feasible for flour, nor do adsorption methods. High-speed centrifuging techniques are suitable for particles of very fine size, but the equipment required is both elaborate and expensive. There remain to be considered many modifications of the technique based on the rate of sedimentation in a liquid medium and measurement by microscopic observations.

Microscopic methods have been extensively studied and described by Work (1928). This procedure is, of course, fundamental in that it gives a direct measure of particle size. However, the preparation of truly representative samples for microscopic observation is difficult, and it therefore becomes necessary to measure a large number of particles, preferably from a replicated series of samples from the material under test. In order to facilitate this type of procedure,

¹ Paper No. 46, Journal Series, General Mills, Inc., Research Laboratories

many investigators have used accessory devices such as the projection of an image of the specimen on a ruled wall screen, or the preparation of greatly enlarged prints of the microscope field.

The use of a sedimentation method requires relatively few assumptions and permits the determination of small particle size without excessive tedium. The fundamental work in the development of this type of procedure was carried out by Odén *et al.* (1916, 1920, 1925, 1926, 1927), who developed the theoretical basis of this method and worked out in detail an apparatus suitable for use with soils.

There are a number of methods available for determining the amount of material that reaches the bottom of a sedimentation column within a given time. Thus Knapp (1931, 1934) devised a very ingenious apparatus based on the determination of changes in hydrostatic pressure immediately above the bottom of the column. This procedure is valid because such pressure decreases in proportion as particles settle below the point of measurement. While the apparatus described by Knapp is elaborate, it has the advantage of providing an automatic photographic record. Odén and coworkers, Calbeck and Harner (1927), and Markley (1934) have described procedures in which the weight of material settling is determined by counterbalancing a pan in the suspension and determining increase in weight as the particles settle out. Calbeck and Harner's paper is of particular value for its discussion of the method of plotting and interpreting the data.

Gründer and Sauer (1937) have devised an optical method for measuring the rate of settling of flour. In one paper they describe apparatus in which a Pulfrich stufenphotometer is used to measure the light transmission of a sedimentation column at various time intervals, stating the precautions necessary for satisfactory results. They show, as might be supposed, that absorption of light depends upon particle size. The same authors later describe a refinement of the Pulfrich stufenphotometer method in which a photronic cell is used. In general, the procedure resembles the visual method but instead of photometer readings one obtains electrical potential measurements in millivolts. The authors found that particles of flour smaller than $40\ \mu$ in diameter settled very slowly and that below this size differences in settling rate were of small magnitude. For this reason they did not make measurements below $30\ \mu$. In their samples the quantity of flour having a particle size greater than $40\ \mu$ constituted 80% of the total and only a small fraction of a percent was smaller than $30\ \mu$. For this reason they felt that measurement of particles having a smaller diameter than $30\ \mu$ could be neglected. The papers by Gründer and Sauer provide a history of the development of a method and in the progress of its development several procedures

are shown to have possibilities. The instrument last named provided for automatic recording of a curve, and by using a standard sample the authors were able to show graphically how far other samples deviated from it.

Cereal investigators have been concerned with the possibility of a correlation between particle size distribution and baking quality. With this purpose in mind, several have devised apparatus aimed at convenience of operation rather than a high degree of precision. Included in this group are Kent-Jones (1939, 1941), Worzella and Cutler (1939), and more recently Chin (1940).

The purpose here is not to describe particle size distributions for particular flour types or to report on the relation of particle size to baking properties. Rather, the intention is to describe the application of sieving, microscopic, and sedimentation techniques to the determination of particle-size distribution in a graded series of flour samples, thus obtaining a direct comparison of results from the three types of measurement. The apparatus employed for sedimentation technique contains certain minor innovations which are believed to contribute materially to increased precision in this type of measurement.

Experimental

Preparation of samples: A bulk lot of farina obtained from a hard red winter wheat was sized by gradual reduction on the smooth rolls of an Allis-Chalmers experimental mill, the product being sifted after each passage through the rolls. In this manner, a graded series of samples, presumably of varying particle-size distribution, was obtained (see Table I).

TABLE I
PREPARATION OF FLOUR SAMPLES

Sample No.	Passed through silk No.	Retained on silk No.
A	8xx	10xx
B	10xx	11xx
C	11xx	13xx
D	13xx	14xx
E	14xx	16xx
F	16xx	—

Microscopic measurements: A Bausch and Lomb Type B micro-projector assembly was employed. Essentially, this apparatus comprises a carbon arc light source, water-cell heat barrier, microscope, and a reflecting prism mounted above the ocular of the microscope in such fashion that the image of the specimen can be thrown on a screen.

On the screen was ruled a grid, the equivalent dimensions of which had been calibrated by the use of a stage micrometer. Mounts were prepared by suspending a few milligrams of sample in several drops of dilute collodion on a microscope slide, stirring vigorously with a fine-pointed glass rod, and allowing the collodion to dry. No cover glass was employed because it was found that rubbing the material under a cover glass tended to bring about a variable amount of wet milling. In the actual determination of particle size, the slide was moved unidirectionally, all particles passing across the grid of the wall screen being counted and their horizontal diameter measured to the nearest $5\ \mu$. Only one diameter was measured for each particle, the manner of measurement being illustrated in Figure 1. The image of the

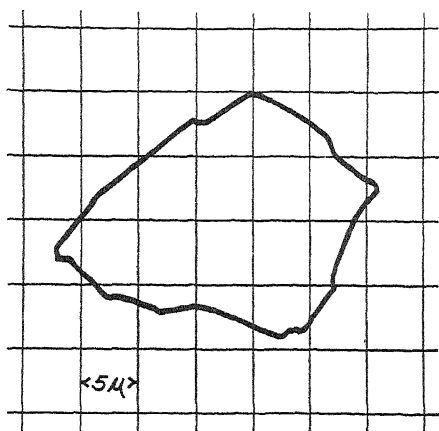


Fig. 1. Method of microscopic measurement of particle size.

particle was located on the screen so that approximately equal areas of the image lay above and below one of the horizontal grid rulings. The distance between edges of the image on this horizontal line was taken as the diameter. This procedure is justified on the assumption that the particles are oriented at random on the slide. Preliminary measurements of both horizontal and vertical diameters gave the same result, indicating the validity of this assumption.

When the full length of the mount had been traversed, the slide was moved at right angles to the first direction just sufficiently to bring uncounted particles into the field, and the process was repeated, in a direction opposite to that first employed, until a sufficient number of particles had been measured.

It was found that a substantial saving in time and effort could be effected by following a procedure suggested by Work (1928). The

particle count was arbitrarily divided into two classifications—for example, into particles larger than $20\ \mu$ and those of smaller size, the latter group containing many times more particles than the former. When a sufficient number (say 250–300) in the small sizes had been counted, the number of particles in each class for the entire distribution was recorded. A second count was then made in which all particles less than $20\ \mu$ in diameter were neglected, continuing this count until the total number was adequate to characterize the size distribution of the larger particles. The ratio of the totals for large particles in the two counts was then determined and the values for the smaller-size classes multiplied by the factor thus obtained. The data in Table II, while not showing a complete count, will serve to illustrate the form of computation employed.

TABLE II
ESTIMATION OF PARTICLE SIZE DISTRIBUTION BY MICROSCOPE
COUNT—SAMPLE B, MOUNT 1

Size class	Number of particles			
	Original count	Second count	Corrected count	Total
μ				
0–5	111	—	1510	1621
6–10	119	—	1618	1737
11–15	27	—	367	394
16–20	19	—	258	277
Subtotal $<20\ \mu$	276	—	—	—
21–25	6	49	—	55
26–30	3	25	—	28
31–35	1	24	—	25
36–40	1	2	—	3
41–45		1	—	1
>46	4	103	—	107
Subtotal $>20\ \mu$	15	204	—	—

The second column of Table II shows values obtained in the first count, in which a sufficient number of particles of 0– $20\ \mu$ in diameter were measured to establish this portion of the distribution. In the second count, only particles above $20\ \mu$ in diameter were considered. The values for the size classes below $20\ \mu$ were then multiplied by the factor $204/15$ to give the results shown in the fourth column. The values in the third and fourth columns were then added to those in the second to give the complete distribution shown in the last column. The size characteristics of all samples were determined in duplicate in this manner, with at least two mounts used for each determination.

Sedimentation measurements: Measurements of particle size by sedimentation techniques are fundamentally based on Stokes' law, which may be expressed thus:

$$V = \frac{h}{t} = \frac{2gr^2(d_1 - d_0)}{9\eta}$$

where V is the rate of settling in cm/second, h the distance of fall in cm, t the time in seconds, g the acceleration due to gravity, r the particle radius in cm, d_1 and d_0 the density of flour and liquid respectively in g/cc, and η the viscosity of the liquid in poises. For convenience, this expression may be rearranged to give:

$$t = \frac{7.5 \times 10^6 h \eta}{gr^2(d_1 - d_0)}$$

where t is now in minutes, r in microns and the other quantities are as previously defined. For any given set of conditions this reduces to:

$$t = \frac{K}{r^2}$$

or

$$r = \frac{K'}{\sqrt{t}}$$

where K' is a constant for that set of conditions.

Thus it is possible to calculate the size of a spherical particle that will settle a given distance in a given time. If it were possible to allow a sample of flour, for example, to settle through a column of liquid, with all flour particles starting from the same level, and if the concentration of particles that had settled a given distance could be determined at various time intervals, one could then calculate directly from such data the size distribution of the flour sample. For a variety of reasons, this procedure is impractical. However, Odén (1916) has shown that such a size distribution may be calculated from data on the rate of settling of a sample homogeneously dispersed throughout the entire settling column.

Briefly, the procedure is this: Plot the weight of particles settled out as ordinate and time as abscissa. Then the intercept on the ordinate of the tangent to the curve at any time t_1 gives the weight of particles of radius equal to, or larger than, that of a particle which will just settle a distance equal to the height of sedimentation column in time t_1 . By determining a number of such points of interception, the size distribution of the sample may be adequately characterized.

The actual measurements of particle-size distribution by the sedimentation technique were carried out in the apparatus shown

schematically in Figure 2. This device, which is essentially the same as that originally suggested by Odén (1916), comprises a sedimentation column in which is suspended a pan attached to the left arm of the analytical balance. The entire assembly, exclusive of the balance, is

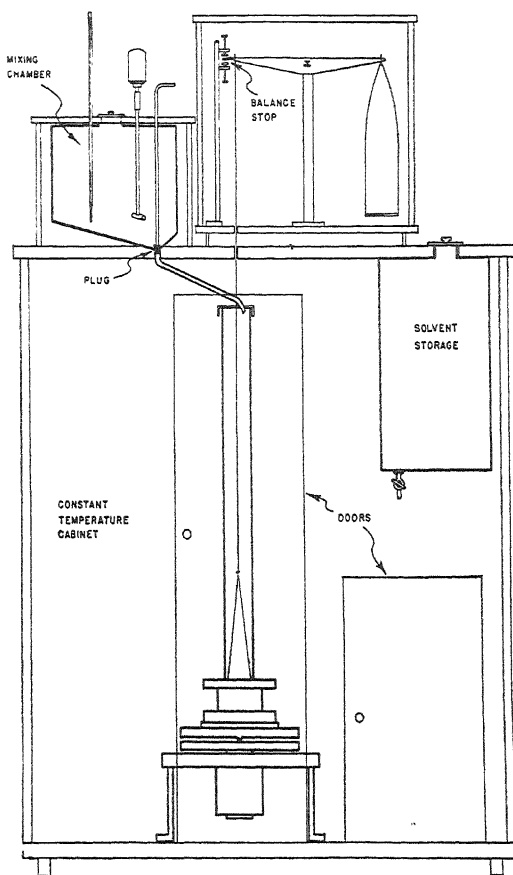


Fig. 2 Apparatus for sedimentation measurements.

contained in a cabinet provided with suitable means for circulation of temperature-controlled air. Above and to one side of the sedimentation column and connected with it by half-inch copper tubing is a small tank provided with an outlet plug, a motor-driven stirrer, and a thermometer. The base of the sedimentation column, shown in detail

in Figure 3, was designed to permit mechanical raising and lowering of the entire column for convenience in removal and also made provision for accurate centering of the pan on which settling particles accumulated.

Relatively close control of temperature ($\pm 0.1^{\circ}\text{C}$) is necessary to prevent variation in density and viscosity of the liquid employed.

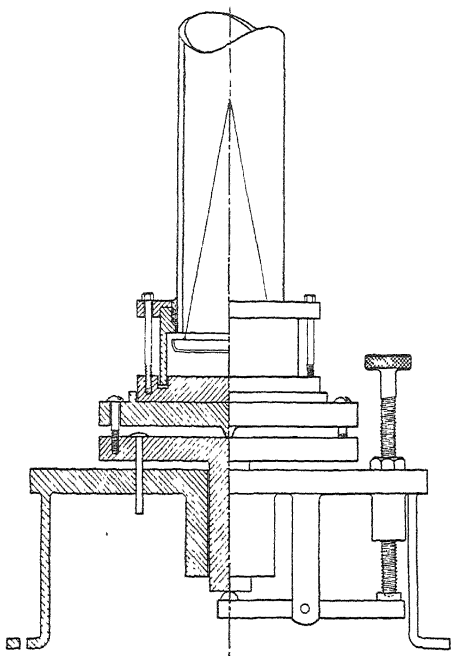


Fig. 3. Detail of base of sedimentation column.

Such temperature control is of even greater importance in preventing the formation of convection currents in the sedimentation column.

Early experiments with a pan smaller in cross-sectional area than that of the sedimentation column were found to give unreliable results, in that the total weight collected on the pan did not agree with the calculated value. Since the density of the flour sample, the density of the liquid employed, and the original sample weight are known, it is possible to calculate the total weight of flour that should settle out on a pan of known area. With pans small enough to fit within the main portion of the sedimentation column, only 60%-80% of the calculated flour weight was accumulated even after long periods of standing.

This discrepancy was not due to incomplete settling but to passage of material through the annular space between the glass tube and the edge of the pan. All attempts to modify the type of suspension employed and to alter the size of pan were unsuccessful until it was found that employing a pan larger in area than the cross section of the sedimentation column and suspending it immediately below the bottom of the column, gave total flour weights of 98%–101% of the calculated values. The reason for this behavior is not known but apparatus of this design has given entirely satisfactory results with many samples.

In carrying out the determination a suitable weight of flour, usually 10 g., was suspended in 2600 ml of liquid in the small tank. The mixture was agitated for several minutes to effect complete dispersal of the flour, the plug was then opened with the stirrer still running, and the dispersion allowed to drain into the sedimentation column. With the 75-mm-diameter glass tube which we employed, this quantity of suspension gave a 60-cm depth of liquid above the balance pan. Zero time was recorded as the time when all the liquid, with the exception of the final slow drainings, had flowed into the sedimentation column. The pan had previously been balanced while suspended in the liquid alone. A known weight (0.1 or 0.2 g) was added to the right-hand balance pan and the time recorded when the pointer swung past the balance point. The beam arrest was raised, another weight increment added to the right-hand pan, and the process repeated. The increments of weight added were varied so that a reading was obtained every four to five minutes until sedimentation was virtually complete, and a final weight reading taken after two to four hours.

Several precautions must be observed. The rate of settling must not be so great that Stokes' law is no longer valid. For ordinary flours and dispersion media, the maximum rate is well within this limiting value but some difficulty may be encountered if attempts are made to measure the size distribution of very coarse materials by this technique. The sample must be homogeneously dispersed throughout the liquid at the time that settling starts and the concentration of sample must be low enough so that the particles fall freely without colliding with each other. Obviously, the sample must be completely dispersed so that no aggregates of particles remain in the suspension, since such aggregates will settle as individual particles of that size.

In the calculation of the particle-size distribution, the accumulated weight was plotted against time on a large graph as illustrated in Figure 4. The times necessary for a series of particles of radii r_1, r_2, r_3 , etc., to settle the full length of the column, were calculated from Stokes' law, as previously described, after determination of the other factors entering into this equation and evaluation of the constant K . The

times thus calculated are shown as crosses on the accumulation curve. The tangent to the accumulation curve was found graphically for each of these times and the intercept of the tangents on the ordinate determined.

It is possible to estimate the tangents to the accumulation curve arithmetically from the difference between accumulated weights corresponding to times t_1 and t_2 , but this procedure is not satisfactory under conditions in which the slope of the curve is changing rapidly. The results obtained by graphical and arithmetic determination of tangents

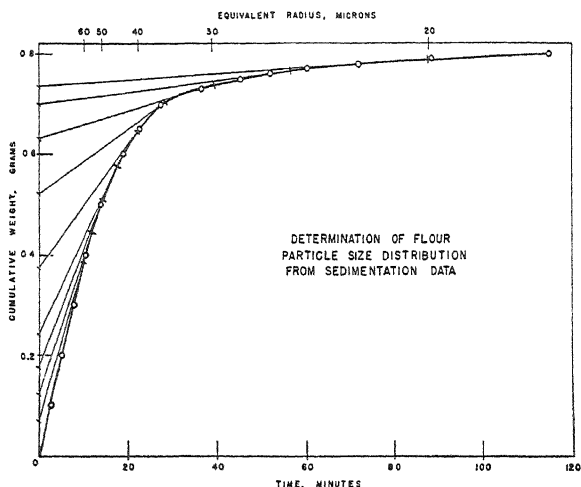


Fig. 4. Determination of flour particle-size distribution from sedimentation data

are shown in Table III. It will be noted that arithmetic determination of the tangents gives values in most size classes in which the apparent weight distribution is shifted toward the smaller sizes. It is believed that careful graphical determination of the tangent at a given point gives the more reliable result.

If an equation could be found that would fit the accumulation curve data satisfactorily, it would obviously be possible to differentiate this equation and thus obtain a satisfactory mathematical expression for the tangent and point of intercept for any given time. It has been our experience, however, that the shape of the accumulation curve changes sufficiently from sample to sample so that no general equation will fit such data with satisfactory precision, and the labor involved in fitting a special, rather complicated equation to each particular set of data is not justified by the resulting increase in precision.

TABLE III
COMPARISON OF RESULTS OBTAINED BY GRAPHICAL AND ARITHMETIC
CONVERSION OF SEDIMENTATION DATA—SAMPLE F

Radius	Graphical		Arithmetic	
	Intercept	$I_k - I_{k-1}$	Intercept	$I_k - I_{k-1}$
μ	g	$\%$	g	$\%$
0	3.27	28.1	3.27	17.7
15	2.35	17.1	2.69	17.7
20	1.79	14.0	2.11	15.0
25	1.33	12.1	1.62	13.7
30	0.94	11.3	1.17	11.0
35	0.56	6.9	0.81	13.4
40	0.34	2.6	0.37	1.8
45	0.26	2.0	0.31	2.4
50	0.19	0.6	0.23	2.7
55	0.17		0.14	
		94.7		95.4

The calculation of settling times noted above requires the determination of density of both the flour and the liquid employed for the dispersion. We have found it convenient to use a pycnometer for both measurements. The density of the liquid is determined in the usual manner. The pycnometer is then cleaned and dried, a small sample of flour introduced, its weight determined by difference, and the vessel carefully filled with the liquid to be employed for the dispersion. After the vessel and its contents come to temperature equilibrium, the volume is carefully adjusted and the total weight determined. From this value is calculated, by difference, the weight of liquid required to fill the pycnometer, and from this value the volume of liquid. The difference between total volume and liquid volume is obviously that of the flour, from which value the flour density may be calculated.

The selection of a liquid to be employed for this type of determination is governed by the density and viscosity of the liquid in addition to certain special requirements. The liquid must not react with the flour in any way. It should not penetrate the flour particles, for such penetration would change the density of the flour, and it should not cause swelling of the flour particles. We have found that various

petroleum fractions may be satisfactorily employed. It is desirable to use a liquid of as low a density as is consistent with reasonable accuracy in the determination of the accumulation curve, since the greater the difference in density between flour and liquid, the smaller will be the error introduced by errors in measurements of density or by change in density with time, or with temperature fluctuation. With petroleum fractions such as gasoline, it has been observed that there is a gradual penetration of liquid into the flour particles with time. Such penetration is sufficient to change flour density by values up to 0.005 g/cc, but this variation is not of sufficient magnitude to cause significant error if the density of the liquid is less than unity.

A comparison of particle-size values given by microscopic and sedimentation measurements with those deduced from data on sieve openings is shown in Table IV. It is evident that the actual particle size

TABLE IV
COMPARISON OF SIZE RANGES FROM SIEVE OPENINGS,
MICROSCOPIC AND SEDIMENTATION DATA

Sample	Sieve openings	Central 80% by weight	
		Microscope range	Sedimentation range
	μ	μ	μ
A	140-193	48-116	41-83
B	125-140	56-116	34-76
C	99-125	47- 96	31-74
E	76- 94	37- 80	20-57
F	— 76	14- 82	11-45

values in all cases are much smaller than would be expected from the sieve openings. Apparently in dry sifting the particles tend to agglomerate, and the sifting behavior is then governed by the size of such aggregates. Accordingly, ultimate particle-size values of flours cannot be determined by sifting.

Typical results obtained by microscopic and sedimentation procedures on the graded series of flour samples previously described are shown in Figure 5. It is apparent that either type of measurement can be used satisfactorily to differentiate between flours, although the absolute results obtained are not the same. In all cases, microscopic measurements gave significantly larger mean particle diameters.

Consideration of the shape of flour particles affords a reasonable explanation for the difference in results. In all except the smallest particles, which are apparently free starch granules, the shape is highly irregular, the particles usually being appreciably larger in one or two dimensions than in the third. As an exaggerated case one may consider the estimation of the size of a platelike particle. In micro-

scopic measurements, such a particle would tend to settle in the mount with the two larger dimensions parallel to the surface of the microscope slide, and it is only these dimensions which are measured. In the determination by sedimentation, however, it is to be expected

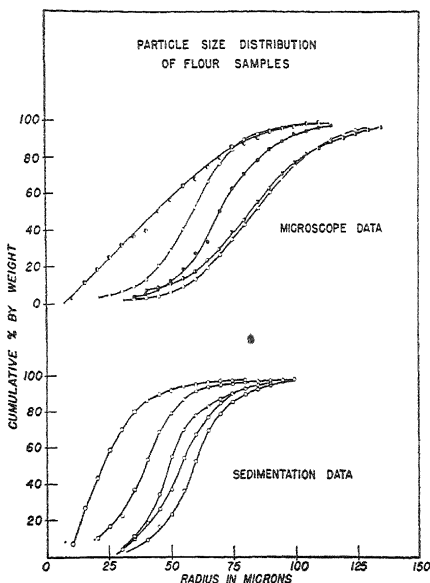


Fig. 5. Particle-size distribution of flour samples.

that the particle would settle with these dimensions parallel to the line of fall. In other words, the settling behavior of this particle would be governed largely by its smaller dimensions. Thus, in the aggregate, microscopic measurement of flour particles tends to give values that are too large and sedimentation measurements values too small. It seems that the best estimate would be the average between data obtained with the two types of measurement. For comparative studies of flours it appears that either could be employed, but the labor involved in microscopic measurements is many times greater than that necessary by the sedimentation technique.

Summary

Determinations of particle-size distribution have been made by both microscopic and sedimentation procedures on a series of flours produced from one bulk lot of farina and sized by sifting.

Values obtained by both microscopic and sedimentation techniques were much lower than would be predicted from the size of sieve openings. Flour particles are therefore not completely deflocculated in sifting, and aggregates rather than individual particles determine behavior.

The microscopic and sedimentation techniques gave different but well correlated results, the larger values being obtained with the former. An explanation is advanced for this discrepancy. True values are believed to lie between those given by the two methods.

Either procedure may be employed, but the sedimentation technique is much less laborious.

Acknowledgment

The authors are indebted to C. B. Kress for preparing the flour samples, to G. M. Burkert for assistance in the microscopic measurements, and to R. I. Derby for preparation of illustrations.

Literature Cited

- Calbeck, J. H., and Harner, H. R. *
1927 Particle size and distribution by sedimentation method. *Ind. Eng. Chem.* 19: 58-61.
- Chin, Kuo Chun
1940 Relations between particle size in flour and dough properties. *Compt. rend.* 210: 581-583.
- Gründer, W.
1937 Der Sedimentograph. *Mühlenlab.* 7: 170-176; *Kolloid-Z.* 80: 287-291; *D.R.G.M.* 1397577.
- and Sauer, H.
1937 Die Ermittlung von Körnungskennlinien und Oberflächen von Zerkleinerungsprodukten am Beispiel Mehl. *Kolloid-Z.* 79: 257-273.
- Kent-Jones, D. W.
1941 Studies in flour granularity. *Cereal Chem.* 18: 358-369.
- , Richardson, E. G., and Spalding, R. C.
1939 Flour granularity. *J. Soc. Chem. Ind.* 58: 261-267.
- Knapp, R. T.
1931 Apparatus for measuring the fineness of finely divided materials. U. S. Patent No. 1,838,628, Dec. 29 (assigned to Riverside Cement Co.).
1934 New apparatus for determination of size distribution of particles in fine powders. *Ind. Eng. Chem. (Anal. Ed.)* 6: 66-71.
- Markley, M. C.
1934 Flour particle size by the sedimentation method. *Cereal Chem.* 11: 654-660.
- Odén, S.
1916 Eine neue Method zur Bestimmung der Körnerverteilung in Suspensionen. *Kolloid-Z.* 18: 33-48.
1920 Die automatisch registrierende Sedimenttiervorrichtung und ihre Anwendung auf einige kolloid-chemische Probleme. *Kolloid-Z.* 26: 100-121.
1925 Om Sedimentationsanalys I. *Tek. Tid. (Bergsvetenskap)* 55: 65-68.
- and Meyer, F.
1926 Om Sedimentationsanalys II. *Tek. Tid. (Bergsvetenskap)* 56: 69-72.
- and Winckler, N.
1927 Om Sedimentationsanalys III. *Tek. Tid. (Kemi)* 57: 16-18.
- Work, L. T.
1928 Particle size determination. *Proc. Amer. Soc. Test. Materials* 28: 771-812.
- Worzella, W. W., and Cutler, G. H.
1939 A critical study of technique for measuring granulation in wheat meal. *J. Agr. Research* 58: 329-341.

THE EFFECT OF STORAGE OF CORN ON THE CHEMICAL PROPERTIES OF ITS PROTEINS AND ON ITS GROWTH-PROMOTING VALUE ¹

D. BREESE JONES, J. P. DIVINE, and CHAS. E. F. GERSDORFF

Protein and Nutrition Research Division,
Bureau of Agricultural Chemistry and Engineering,
U. S. Department of Agriculture

(Received for publication June 1, 1942)

Since large quantities of wheat, corn, and other grains are stored over long periods in warehouses and granaries of various types under the Federal ever-normal granary program, any information on the extent and nature of deteriorative changes that occur in stored grain is of special value. Studies of the kind, rate, and extent of storage changes in grain should yield information that will aid in determining the type of bins needed and storage conditions that will result in minimum deteriorative changes. Also a knowledge of the specific kinds of changes that occur may suggest the best use for grains that have deteriorated in storage—whether for food or for other purposes. The results of tests herein reported, while not extensive enough to justify specific recommendations, nevertheless throw additional light on some aspects of grain storage.

In a previous paper (Jones and Gersdorff, 1941) it was shown that when wheat and its milling products are stored, marked changes occur in the properties of the proteins. These changes include (1) decrease in the solubility of the proteins in various dispersing agents, such as neutral salt solutions and alcohol; (2) proteolysis, or breaking down of the native proteins into entities of smaller molecular dimensions; and (3) decrease in digestibility when treated with pepsin and trypsin *in vitro*. The extent of the changes depends on temperature, type of container, and on whether the whole-wheat kernels or their milling products are stored. The present paper describes the effect of similar storage on the chemical properties of the proteins of corn and on the growth-promoting value of stored corn as determined by feeding experiments with albino rats.

The results of the studies on corn here presented show that during storage marked changes occur in the properties of corn proteins. They also indicate a decrease in growth-promoting value of corn as a result of storage. The protein chemical changes are largely of the same order, rate, and extent as those previously found for wheat and wheat flour stored under the same conditions as those here described for corn.

¹ Preliminary notes on the effect of storage on the proteins of whole and ground seeds have been previously published (Jones and Gersdorff, 1938 and 1939).

Chemical Effects

Material: The shelled Yellow Dent corn used in these studies was grown on the U. S. Department of Agriculture experimental farm at Arlington, Virginia, and was obtained through the kindness of Dr. Merl T. Jenkins of the Division of Cereal Crops and Diseases, Bureau of Plant Industry. Immediately after harvesting, the mature corn was dried on the cob at 89°F in an open room in an air current generated by electric fans. The dried, shelled corn was delivered September 30, 1938.

Analysis of the corn a few days after receipt gave the following results: moisture 10.59%, nitrogen 1.87%, and fat 4.77%. (The figures for nitrogen and fat are on a moisture-free basis.)

Preparation and storage of the material: A portion of the corn was first cracked in an electric-driven Hobart mill, then ground coarsely, and finally reduced to a fine meal (October 4, 1938).

On October 5, 1938, samples of the ground corn in air-tight Mason jars and in closely woven cotton bags were stored in dark cupboards in a room maintained at a temperature of $76^{\circ} \pm 3^{\circ}$ or 4° F and at a relative humidity of about 55%. Similar samples were stored in a refrigerating room maintained at a temperature of 30°F. Each jar of the ground corn contained enough material to provide for the required analyses at the end of the various storage periods. Samples of the whole kernels were also stored in two-quart Mason jars at 76°F and 30°F.

Analyses: Analyses of the ground corn samples stored in jars were made at the end of 1, 3, 6, 12, and 24 months of storage, and of those stored in bags at the end of 6, 12, and 24 months. Portions of the samples of whole-corn kernels were ground to the approximate fineness of the corn meal and analyzed after storage periods of 12 and 24 months.

Procedure: The various analyses made on the stored samples were chosen as suitable for revealing the nature and extent of changes in the properties of the proteins that might occur during storage. Proteins readily undergo changes of three common and well known kinds when subjected to the action of chemical and physical agents. One of these is degradation through hydrolysis or proteolysis, characterized by progressive breaking down of the protein into products of smaller molecular size, namely peptides of decreasing dimensions and finally amino acids. The degradation of protein is commonly accomplished by the action of acids or alkalis, and by proteolytic enzymes. Another kind of change, *denaturation*, is commonly characterized by decrease in solubility of the proteins in reagents which readily disperse them in their native or unchanged state. There may also be *changes*

in digestibility of proteins. It is known that the digestibility of many proteins is affected by the action of heat, light, alcohol, and other agents.

To learn the extent of denaturation during storage, changes in solubility of the proteins were determined by periodically extracting portions of the samples with 3% NaCl solution, 70% alcohol, and 3% sodium salicylate solution, and determining total nitrogen in the extracts.

The extent of proteolysis was measured by determining the "true-protein value" (protein nitrogen) according to the copper hydroxide method of Stutzer (1881). By means of this method the native unchanged protein is converted into an insoluble copper derivative which can be separated from the break-down products.

Digestibility was measured by the amount of protein (as represented by nitrogen) rendered soluble by digesting the samples with pepsin and trypsin.

Determinations of moisture, total nitrogen, and free ammonia nitrogen also were made on the fresh material, and on the samples at the end of the different storage periods.

The first analyses were made on the fresh samples at the time they were placed in storage. The results of these analyses served as a basis of comparison with similar data obtained on the stored samples. Throughout the investigation special care was taken to have all the corresponding analyses made in the same way and under the same conditions in order to have the results of the different sets of analyses as comparable as possible. The samples of stored corn kernels were ground to a meal in a hand-driven mill just before analysis, care being taken to have the material reduced to the same degree of fineness for each set of analyses.

The details of the methods used in carrying out the different analyses and procedures have been described in a previous paper on the effect of storage on the proteins of wheat and wheat flour (Jones and Gersdorff, 1941).

Discussion of chemical effects: At no time during the storage periods was there any visible indication of spoilage or deterioration of the material. No evidence of mold or insect infestation was apparent in the samples used for chemical tests. Some of the ground-corn samples stored in bags at 76°F had developed a slightly sharp odor, and those stored at 30°F had acquired a little "off" odor due to absorption from other material stored in the refrigerating room.

The results of the analyses of the ground corn and of the corn kernels made at the time they were placed in storage and at the end of the storage intervals are shown in Tables I and II. These results are

TABLE I
EFFECT OF STORAGE ON THE PROTEINS OF GROUND CORN
(Results expressed in milligrams per 100 grams material)

Determinations	Values for the fresh material	Months at 30° F.					
		1	3	6	12	24	
GROUND CORN STORED IN JARS							
Moisture	10,590	10,620	10,630	10,590	10,800	10,590	
Total nitrogen	1,870	1,870	1,870	1,870	1,870	1,870	
True protein nitrogen	1,530	1,478	1,380	1,359	1,327	1,280	
Free ammonia nitrogen	36.8	36.5	36.1	36.8	36.5	36.8	
Nitrogen soluble in 3% NaCl	462	368	357	347	331	312	
Nitrogen soluble in 70% alcohol	476	350	301	287	277	263	
Nitrogen soluble in 3% sodi- um salicylate	714	620	557	494	451	410	
Nitrogen rendered soluble by peptic-tryptic digestion	1,775	1,767	1,746	1,688	1,638	1,618	
Determinations	Values for the fresh material	Months at 76° F.					
		1	3	6	12	24	
Moisture	10,590	10,620	10,630	10,620	10,860	10,630	
Total nitrogen	1,870	1,870	1,870	1,870	1,870	1,880	
True protein nitrogen	1,530	1,464	1,324	1,219	1,186	1,112	
Free ammonia nitrogen	36.8	36.1	36.1	36.8	36.8	37.2	
Nitrogen soluble in 3% NaCl	462	347	326	294	263	232	
Nitrogen soluble in 70% alcohol	476	336	287	259	229	210	
Nitrogen soluble in 3% sodi- um salicylate	714	515	462	389	306	249	
Nitrogen rendered soluble by peptic-tryptic digestion	1,775	1,724	1,702	1,624	1,537	1,444	
Determinations	Values for the fresh material	Months at 30° F.			Months at 76° F.		
		6	12	24	6	12	24
GROUND CORN STORED IN BAGS							
Moisture	10,590	13,100	12,850	14,120	9,970	10,460	9,930
Total nitrogen	1,870	1,880	1,880	1,890	1,870	1,870	1,870
True protein nitrogen	1,530	1,313	1,270	1,243	1,135	1,098	1,034
Free ammonia nitrogen	36.8	36.8	37.0	37.0	36.5	36.8	37.0
Nitrogen soluble in 3% NaCl	462	326	308	301	252	229	188
Nitrogen soluble in 70% alcohol	476	265	258	242	231	210	189
Nitrogen soluble in 3% sodi- um salicylate	714	420	341	289	326	286	235
Nitrogen rendered soluble by peptic-tryptic digestion	1,775	1,638	1,595	1,533	1,458	1,386	1,257

expressed in milligrams per 100 g of the fresh and stored material, and are calculated to a moisture-free basis. For convenience in comparing and visualizing the changes at different periods and under different conditions of storage some of the results shown in Tables I and II are summarized in Table III, in which the figures represent decreases calculated as percentages of the values found for the fresh material.

TABLE II
EFFECT OF STORAGE ON THE PROTEINS OF CORN KERNELS
(Results expressed in milligrams per 100 grams material)

Determinations	Values for the fresh material	Stored in jars			
		Months at 30°F		Months at 76°F	
		12	24	12	24
Moisture	11,360	11,360	11,360	12,030	11,450
Total nitrogen	1,870	1,870	1,880	1,870	1,880
True protein nitrogen	1,530	1,416	1,373	1,341	1,275
Free ammonia nitrogen	36.8	36.7	37.0	36.6	36.8
Nitrogen soluble in 3% NaCl	462	383	377	362	343
Nitrogen soluble in 70% alcohol	476	347	335	321	288
Nitrogen soluble in 3% sodium salicylate	714	537	515	489	435
Nitrogen rendered soluble by peptic-tryptic digestion	1,775	1,751	1,734	1,742	1,710

The values for total nitrogen and ammonia nitrogen remained practically constant in all of the samples. Moisture content did not change, except in the case of the material stored in bags. All of the other determinations given in Tables I and II show progressive changes when compared with similar determinations at the ends of preceding periods of storage. The samples stored in bags which permitted access of air showed greater changes than did those stored in air-tight jars. Temperature was also an important factor. Although significant changes occurred in the samples stored at 30°F, they were less than those in the samples stored at 76°F. The effect of storage was much greater on the ground corn than on the unground kernels.

An inspection of the tables will show that the most striking change was the marked decrease in solubility of the proteins.

Another extensive change that took place during storage was a progressive breaking down of the native protein into smaller fragments. This change was measured by determining the so-called true-protein content by the copper hydroxide method. As in the case of the solubility changes, the decreases in true-protein values were greater in the samples stored in bags than in those stored in jars, and greater

TABLE III

EFFECT OF STORAGE ON THE PROTEINS OF GROUND CORN AND CORN KERNELS
(Results expressed as percentage decreases)

Determinations	Months at 30°F				Months at 76°F			
	1	3	12	24	1	3	12	24
GROUND CORN STORED IN JARS								
True protein nitrogen	3.4	9.8	13.2	16.3	4.3	13.5	22.4	27.3
Nitrogen soluble in 3% NaCl	20.1	22.7	28.3	32.5	24.9	29.4	43.1	49.8
Nitrogen soluble in 70% alcohol	26.5	36.7	41.8	44.7	29.4	39.7	51.9	56.0
Nitrogen rendered soluble by peptic-tryptic digestion	0.4	1.6	7.7	8.8	2.8	4.1	13.4	18.6
GROUND CORN STORED IN BAGS								
		6	12	24		6	12	24
True protein nitrogen	—	14.1	17.0	18.7	—	25.7	28.2	32.4
Nitrogen soluble in 3% NaCl	—	29.4	33.3	34.8	—	45.4	50.4	59.3
Nitrogen soluble in 70% alcohol	—	44.3	45.8	49.2	—	51.4	55.9	60.3
Nitrogen rendered soluble by peptic-tryptic digestion	—	7.7	10.1	13.6	—	17.8	21.9	29.2
CORN KERNELS STORED IN JARS								
			12	24			12	24
True protein nitrogen	—	—	7.4	10.3	—	—	12.3	16.6
Nitrogen soluble in 3% NaCl	—	—	17.1	18.4	—	—	21.6	25.7
Nitrogen soluble in 70% alcohol	—	—	27.1	29.6	—	—	32.5	39.5
Nitrogen rendered soluble by peptic-tryptic digestion	—	—	1.4	2.3	—	—	1.8	3.6

when stored at 76°F than at 30°F. Changes in the true-protein values of the stored corn kernels, like the solubility changes, were less than in the stored ground corn, amounting to only a little over half as much.

Decrease in solubility of the proteins in dispersing agents (denaturation) during storage, and increase in soluble nitrogen due to proteolysis as measured by the Stutzer copper hydroxide method, represent two types of changes producing opposite effects. The substantial over-all decrease in protein solubility probably is, therefore, the resultant of two types of protein alteration, one tending toward an increase in soluble nitrogen and the other toward a decrease, with the latter predominating. Accordingly, it might be concluded that the extent of protein denaturation was actually greater than is indicated by the solubility data.

Decreases in digestibility were observed in all of the stored samples, the extent varying according to the type of container and storage temperature. The changes in the corn kernels, however, were small.

The rapid rate at which the changes occurred during the early periods of storage deserves emphasis. Decreases in true protein and in solubility were much more rapid during the early storage periods than later. Ground corn stored in bags changed even more rapidly than the samples stored in jars. The changes in the corn kernels also apparently developed more rapidly during the first few months of storage than later. Analyses were not made on the stored kernel samples until after 12 months' storage.

The effects of storage on the proteins of corn were very similar in type and extent to those previously described for wheat and wheat flour stored under practically identical conditions. Decreases in solubility and digestibility and the extent of protein breakdown were about of the same order in wheat and corn. The changes were much greater in the flour than in the wheat kernels, and greater in the samples stored in bags than in those stored in jars. The effect of storage was also much more marked during the early storage intervals than later. The changes that occurred during the first month of storage were in some instances as much as three-fourths of those found at the end of 24 months.

The probable causes of the changes occurring in the proteins of wheat during storage were discussed in the publication already referred to, in which the changes were ascribed to the effects of enzymic action and oxidation. Since the storage effects on the proteins of corn are so similar to those on the wheat proteins, it seems highly probable that they are attributable to the same causes.

Nutritional Effects

Concurrently with the chemical studies, feeding experiments with albino rats were conducted in order to see what effect, if any, storage might have on the nutritive value of the proteins of corn. The materials used and the conditions of storage were the same as those already described under "Chemical Effects."

About 80 pounds of corn was ground to a fine meal in an electrically driven mill, care being taken to avoid heating. One-half of the meal was placed in two-quart Mason jars (made air-tight with rubber rings) and stored in a dark cupboard in an air-conditioned room maintained at 76°F and 55% relative humidity. The remaining half of the meal was placed in cotton bags and stored under similar conditions. Whole corn kernels, in two covered galvanized iron cans sealed with adhesive

tape, were also stored at 76°F. The corn, freshly ground, contained 10.42% protein ($N \times 6.25$) uncorrected for moisture.

With only one exception there was no indication of mold, insect infestation, or of any apparent deterioration in the samples during storage as indicated by "off" odor or color. One sample of corn kernels stored in a can became infested with weevils. The results obtained with this sample (Lot 201, Table IV) are, therefore, invalid.

TABLE IV

EFFECT OF STORAGE ON THE GROWTH-PROMOTING VALUE OF GROUND CORN AND OF WHOLE CORN KERNELS

Lot No.	Storage conditions	Storage periods	Initial weight	Final weight	Food consumption	Gain in 42 days	Gain per g protein eaten
		<i>mos</i>	<i>g</i>	<i>g</i>	<i>g</i>	<i>g</i>	<i>g</i>
93	Fresh unstored corn	—	56	140	551	84	1.52
99	Fresh unstored corn	—	55	138	542	83	1.53
124	Meal in jars at 76°	6	57	120	415	63	1.52
125	Meal in bags at 76°	6	58	126	446	68	1.52
149	Meal in jars at 76°	12	57	113	414	56	1.35
150	Meal in bags at 76°	12	58	111	393	53	1.35
202	Meal in jars at 76°	24	57	114	419	57	1.36
201	Meal in bags at 76°	24	56	110	409	54	1.32
151	Kernels in can at 76° ¹	12	57	103	394	46	1.17
203	Kernels in can at 76°	24	56	112	448	56	1.25

¹ This sample contained weevils.

Feeding tests on the freshly ground corn were started within a day or two after grinding. Subsequent tests were started on the ground corn samples at the end of storage intervals of 6, 12, and 24 months. The stored whole kernels were fed at the end of storage intervals of 12 and 24 months.

Young albino rats (8 to each lot), weighing 55 to 60 g each and equally distributed as to sex and litter mates, were fed the rations over 42-day periods. The animals were weighed twice weekly. On each weighing day the unconsumed feed was discarded, the cups were replenished with fresh diet, and the weight gains and food intake of the animals were recorded.

The diet consisted of 96 parts corn, 3 parts salt mixture (Osborne and Mendel), and 1 part cod-liver oil. It contained 10% of protein, all of which was supplied by the corn. A solution of vitamins, freshly prepared each week, was given orally to each rat by means of a syringe. Two-tenths ml of this solution given daily contained 10 μ g thiamin, 10 μ g riboflavin, 0.05 mg nicotinic acid, 25 mg "Ryzamin B,"² and 15 mg sucrose.

² An extract of rice polishings obtained from Burroughs, Wellcome and Co.

Discussion of nutritional effects: The results of the feeding experiments are shown in Table IV. The figures given for each lot are the averages of fairly closely agreeing values obtained with 8 rats. The data show significant decreases in weight gains as the length of time during which corn or meal was stored increased up to 12 months.

The average gain in weight of rats in Lots 124 and 125 fed the meal stored 6 months in jars and in bags amounted to only 63 g and 68 g, respectively, as compared with 84 g for rats in Lot 93 fed the fresh unstored material. These values represent decreases of 25% and 19% in average weight gains of rats fed stored material as compared with those fed fresh material. The gain in weight per gram of protein eaten, however, was the same (1.52 g), but the consumption of the stored meal was definitely less. The meal may have become less palatable as a result of storage. The decrease in weight gains could have been caused by the lower food consumption rather than by an actual decrease in nutritive value. Either case would represent a deterioration from the standpoint of feeding value.

With meal stored similarly for 12 months, the values for average weight increases for rats in Lots 149 and 150 were 33% and 37% less than that for rats fed the fresh unstored meal (Lot 93). Although consumption of the stored meal was less than that of fresh meal, nutritive value was also less, since the gain per gram of protein eaten was less by about 11%. The average gain in weight of rats in Lots 202 and 201 fed meal that had been stored for 24 months remained essentially the same as for those fed meal stored for 12 months (Lots 149 and 150). The variations shown are considered within the limit of experimental error. With meal that had been stored longer than 6 months the decreases in average weight gain were somewhat greater for the samples stored in bags than for those stored in jars.

The effect of storage on the growth-promoting value of the proteins of whole corn kernels over a storage period of 24 months (Lot 203) was the same as that found for the ground corn stored for the same period. Unfortunately, weevils got into the sample stored for 12 months (Lot 151), which probably accounted for the low average growth increment of 46 g.

A comparison of the effect of storage on the chemical properties of the proteins with that upon their growth-promoting value is shown graphically in Figure 1. An interesting similarity in the profiles lies in the fact that the greater part of the changes occurred during the early periods of storage. About 75% of the decrease in average weight gain at the end of 24 months had already occurred by the end of the first 6-month storage period in the meal stored in jars at 76°; the corresponding ratio for the meal stored in bags was about 50%.

Similarly, the corresponding ratios for decreases in solubility in NaCl, true protein, and digestibility for the meal stored in bags were about 75%, 43%, and 60%, respectively.

It should be emphasized that the corn used in these studies, as already noted, was carefully dried, shelled, and placed in storage very soon after it had been harvested. If the same corn after harvesting had been shocked and allowed to remain in the field over a period of time, as is the common farm practice, a considerable part of the changes observed at the end of the first month's storage period would probably have already taken place.

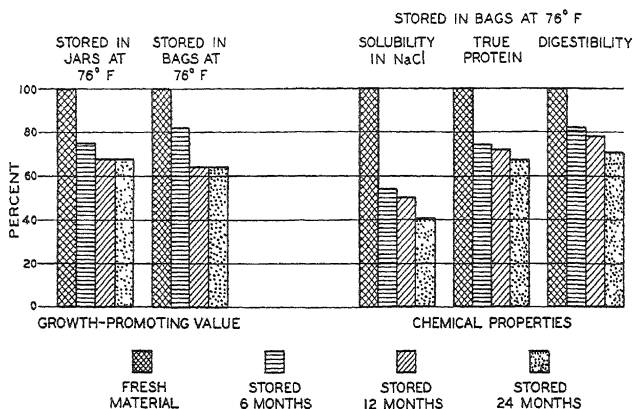


Fig. 1. Decrease in growth-promoting value and changes in chemical properties of the proteins of ground corn as a result of storage for various periods.

The cause of the decrease in growth-promoting value of the corn proteins on storage is not clear. The nature of the chemical changes observed throws little, if any, light on this question. Denatured proteins as a rule are more digestible than native proteins, and proteolysis is one of the first steps involved in protein digestion in the alimentary tract. The fact that the food consumption by rats fed with fresh corn was much higher than that by rats fed with stored material suggests that some change occurred that decreased palatability. It is to be noted that during the storage periods there was a marked progressive increase in free fatty acids as determined in the oil extracted from samples of the stored corn. It does not seem, however, that the decrease in weight gains can be wholly accounted for by impaired palatability. As shown in Table IV there was also a decrease in gain in weight per gram of protein eaten after the first six months of storage. The ease with which proteins are known to be

altered by relatively mild influences suggests that the same factors that brought about the observed chemical changes in the properties of the proteins also could have impaired the integrity of one or more of the nutritionally essential amino acids, or rendered them unavailable for assimilation.

Summary

The effects of storage of ground corn and of whole shelled corn upon the properties of the proteins and upon nutritive value were determined at various intervals over a storage period of two years. The results show that three different types of alterations in the proteins occur: (1) a decrease in the solubility of the proteins, (2) a partial breakdown of the proteins, indicated by a decrease in true protein content, and (3) a decrease in digestibility.

The extent of the alterations is influenced by temperature, type of container, duration of storage, and the nature of the material stored. Samples stored at 76°F were affected more than those stored at 30°F, and those in bags more than those in sealed glass jars. Changes in the ground corn were greater than those in the whole shelled corn. The total nitrogen and free ammonia remained unchanged. The extent of denaturation of the proteins was measured by determination of solubility in 3% NaCl solution, 70% alcohol, and 3% sodium salicylate. At the end of two years' storage in a bag at 76°F the solubility of the ground-corn protein in NaCl solution and in alcohol was approximately 60% less than that of the fresh material. The true-protein value and digestibility had decreased 32% and 29%, respectively. The rates of the decreases were much more rapid during the early storage intervals than later.

Significant decreases in feeding value were also found. During a 42-day feeding period the average gain in weight of albino rats fed with ground corn which had been stored for 6 months in jars at 76°F was 63 g in comparison with 84 g for rats fed with the fresh material, and 56 g for rats fed with material stored for 12 months. The value remained the same at the end of the second year's storage. In every instance less of the stored material than of the fresh material was consumed by the same number of rats in the same time. For material stored 6 months, the gain in weight per gram consumed was the same as for fresh material. For material stored 12 and 24 months, the gain in weight per gram consumed was less.

Literature Cited

- Jones, D. B., and Gersdorff, C. E. F.
1938 Changes that occur in the proteins of soybean meal as a result of storage.
J. Am. Chem. Soc. 60: 723-725.

- 1939 The effect of storage on the protein of seeds and their flours. Soybeans and wheat. *J. Biol. Chem.* **128**: xlix.
1941 The effect of storage on the protein of wheat, white flour, and whole wheat flour. *Cereal Chem.* **18**: 417-434.
Stutzer, A.
1881 Untersuchungen über die Verdaulichkeit und die quantitative bestimmung der Eiweissstoffe. *J. Landw.* **29**: 473.
-

CHEMICAL FACTORS AFFECTING THE BAKING QUALITY OF DRY MILK SOLIDS. II. THE EFFECT OF MILK ON GLUTEN FRACTIONATION ¹

U. S. ASHWORTH and HERBERT A. HARLAND ²

Division of Dairy Husbandry, Agricultural Experiment Station, Pullman, Washington

(Received for publication May 26, 1942)

Almost all dry milk solids now being produced are of good baking quality (Ashworth, Golding, Farrah, and Miller, 1942). However, an occasional sample is found that produces poor loaf volume. An important step toward the correction of this difficulty would be that of finding the nature of the effect of the milk on the dough.

There is no doubt that the effect of proteolytic enzymes is of major importance in determining the quality of bread (Balls and Hale, 1936, 1936a; Jørgensen, 1936). However, the amount of proteolysis ordinarily encountered in baking is so small that no suitable method of measurement, short of the baking itself, is known. Recent reports on the methods for determining the amount of proteolytic enzymes in flour are those of Hildebrand (1940) and Landis (1941).

The method of gluten dispersion in sodium salicylate solution developed by Rose and Cook (1935), McCalla and Rose (1935), Harris (1937, 1938, 1939), and Harris and Johnson (1939, 1940, 1941) seems to be one of the most sensitive found for determining the effect of proteolytic activity. We have used two modifications of the method to furnish evidence of the nature of the action of milk on flour gluten and the effect of milk on the action of papain.

Methods and Materials

The methods of Harris and Johnson (1940) with slight modifications were followed in the first part of this investigation. The dough contained 100 g flour, 3 g yeast, 1.5 g sodium chloride, 3.5 g sucrose, and water as required. The milk doughs contained 6.0 g of dry milk solids. Four mg of Merck's papain was added to one-half of the

¹ Scientific Paper No. 525, College of Agriculture and Agricultural Experiment Station, State College of Washington.

² American Dry Milk Institute Research Grant and in cooperation with Washington State Dairy Products Commission.

doughs just before mixing. They were mixed one minute and fermented one hour.

Following fermentation the doughs were washed with 0.1% phosphate buffer (Dill and Alsberg, 1924), and the gluten dispersed in 10% sodium salicylate solution by gentle agitation on a mechanical shaker for 22 hours. The undispersed gluten and starch were removed by centrifuging. The nitrogen concentration of the dispersion was determined and adjusted to 4.0 mg per milliliter of dispersion. A fraction of the gluten was precipitated by the addition of 1.000 g of magnesium sulfate to 25 ml of dispersion. After standing one hour the precipitate was centrifuged and washed with 10% sodium salicylate containing 1.000 g of magnesium sulfate per 25 ml. The nitrogen content of the precipitate was then determined.

The effect of dry milk solids, in both the presence and the absence of 4 mg % of papain, on the amount of protein nitrogen precipitated by magnesium sulfate solution was studied in the first part of the investigation.

For the second part of the investigation 2.000 g of flour was weighed into 50-ml centrifuge tubes. Five ml of water was added and after thorough stirring the tubes were allowed to digest for 4 hours at 37°C. The digesting mixtures were stirred at 30-minute intervals. Milk, papain, and yeast, when added, were first suspended in the water. All additions made were calculated as a percentage of the flour used. The amounts of papain added were 5 and 50 mg per 100 g of flour. However, since 1.7 times as much water was used in the fractionation of the gluten as could be used in baking, this made the effective concentration of papain, as compared to that in the baking formula, 3 and 30 mg respectively. When yeast was used its amount was 3% added with 5% sucrose and 1.5% salt.

At the end of the digestion period 30 ml of buffer solution (Dill and Alsberg, 1924) was added and the mixture thoroughly agitated. The gluten was allowed to settle for 15 minutes. Then 25 ml of the supernatant liquid was carefully poured off into a graduated cylinder. Four more washings were made with 25-ml portions of the buffer and one with distilled water, with 15 minutes between washings allowed for the gluten to settle. Before the final wash water was decanted, the mixture was centrifuged to pack the gluten in the bottom of the tube; then all the wash water was poured off. A nitrogen determination was made on the combined washings. Also the nitrogen content of all the additions made (such as milk, yeast, and enzymes) was determined so that their nitrogen content could be subtracted from the nitrogen content of the washings. The difference was assumed

to be the fraction of the flour nitrogen soluble in the phosphate buffer at pH 6.8.

For the second fraction 20 ml of 10% sodium salicylate was added to the gluten remaining in the centrifuge tube. The gluten was thoroughly mixed in the dispersing agent with a glass rod and agitated at 15-minute intervals for one hour. After standing overnight the mixture was shaken once and after 30 minutes more was centrifuged 20 minutes at 2600 rpm. The dispersed phase was poured into a Kjeldahl flask for nitrogen determination.

Finally the remaining gluten in the centrifuge tube was quantitatively transferred to another Kjeldahl flask and its nitrogen content determined. This constituted the insoluble residue fraction.

To check the method the sum of the three nitrogen fractions was compared with the nitrogen content of the flour. Agreement was almost invariably within 1% of the total nitrogen content of the flour. Results showing any greater discrepancy were discarded.

Three flours were used in this investigation, flours A, B, and C, containing 14.9%, 13.7%, and 10.8% protein respectively (13.5% moisture basis). Flour A was used in the first part and flours B and C in the second part. The fresh skim milk was pasteurized skim milk secured from the college dairy. The boiled skim milk was this same milk heated in a boiling water bath to 96°C for five minutes. The dry milk solids were commercially produced by the spray process from skim milk which had been preheated before drying.

Discussion of Results

The data obtained in the first part of this investigation are summarized in Table I. Each value for the "basic formula" is the average of four replicate determinations, and each value for the milk

TABLE I
THE EFFECT OF DOUGH CONSTITUENTS ON THE PERCENTAGE OF NITROGEN
PRECIPITATED BY MAGNESIUM SULFATE FROM A DISPERSION OF
THE GLUTEN IN 10% SODIUM SALICYLATE SOLUTION

Description of dough	Nitrogen precipitated by magnesium sulfate with		Differences
	No papain	4 mg % papain	
	%	%	
Basic formula	52.1±0.23	46.1±0.36	6.0*
Basic formula +6% dry milk solids of good baking quality	45.8±0.24	38.6±0.12	7.2*
Basic formula +6% dry milk solids of poor baking quality	46.6±0.39	38.9±0.51	7.7*

* These values lie beyond the 1% level of significance.

formulas represents the average of six different samples of dry milk solids. The standard errors accompany the mean values in the table. These data indicate the degree of degradation of the gluten during mixing and one-hour fermentation of the doughs. Less protein nitrogen is precipitated from the sodium salicylate dispersions of gluten by a given quantity of magnesium sulfate when there has been a reduction in the size of the gluten micelle.

Papain significantly decreased the amount of protein precipitated both with and without milk in the dough formula. The quantitative data show that the addition of milk to the dough formula does not affect the action of papain on the gluten. Since the values in the table under the heading of differences are not significantly different from each other, milk did not activate the papain. It may be noted from the data that the milk and papain had an additive effect when used together. It is purely coincidental that 6% dry milk solids produced about the same amount of degradation of the gluten as 4 mg % of papain. The differences in the amounts of protein precipitated from gluten prepared from dry milk solids of good and poor baking quality are not significant.

Although the quantitative data suggest a similarity in the behaviors of dry milk solids and papain, the physical characteristics of the two glutes were very different while being washed from the other dough ingredients. The papain had a liquefying effect on the gluten, while the milk seemed to have a weakening and coagulating effect.

Table II summarizes the results of the fractionation of gluten by the second modification. The data are given as the percentage of the total nitrogen of the flour. They are arranged in the order of decreasing values for the percentage of the total nitrogen found in the insoluble residue. The concentrations of papain given in the table as 5 and 50 mg per 100 g of flour should be corrected for the amount of water used in this modification. Since the doughs used for gluten fractionation contained 1.7 times as much water as those used for baking, the effective concentrations of papain were only 3 and 30 mg %, respectively, in the doughs used for gluten fractionation. The nitrogen values for any additions made were subtracted from the nitrogen content of the wash-water fraction since they were all water-soluble. Of all the combinations of the various dough ingredients tried, only those in which yeast is included could be actually used in baking. The position of "no additions" in the table leads one to believe that each treatment above it in the table exerts a stabilizing effect on the dough since the amount of insoluble residue is greater. Yeast and the yeast + milk combinations were particularly effective in this stabilizing effect. Even a small amount of papain failed to

TABLE II
NITROGEN DISTRIBUTION OF FLOUR

Additions made (DMS = dry milk solids)	No. of samples	Percent of total nitrogen in:		
		Wash water	Sodium salicylate	Insoluble residue
		%	%	%
FLOUR B				
Yeast + 5 mg % papain + DMS	8	19.3±0.21	24.6±0.78	56.1±0.92
Yeast alone	4	19.5±0.29	24.5±0.37	55.9±0.39
Yeast + 5 mg % papain + fresh milk	4	16.9±0.29	29.5±0.52	53.6±0.62
Yeast + 5 mg % papain + boiled milk	3	20.3±0.29	26.2±2.06	53.5±1.54
5 mg. % papain + DMS	8	27.9±0.08	20.1±0.25	52.1±0.38
Yeast + DMS	7	18.6±0.31	31.7±0.66	49.8±0.14
Boiled milk alone	4	26.7±0.73	23.9±1.14	49.4±1.29
5 mg % papain alone	8	22.0±0.44	29.6±0.84	48.3±1.16
5 mg % papain + boiled milk	4	25.6±0.60	26.6±0.98	47.8±1.73
No additions	8	21.5±0.14	31.3±1.28	47.4±1.65
5 mg % papain + fresh milk	4	27.8±0.89	25.2±0.42	47.1±1.31
Fresh milk alone	4	29.8±0.65	24.6±0.63	45.6±1.44
Yeast + 5 mg % papain	8	20.1±0.12	35.0±1.14	44.9±1.07
DMS alone	18	26.2±0.23	29.3±0.58	44.6±1.00
50 mg % papain + boiled milk	4	39.6±0.40	48.1±0.19	12.1±0.33
50 mg % papain + fresh milk	4	35.6±0.85	52.5±0.40	11.9±0.69
Yeast + 50 mg % papain + fresh milk	4	54.7±0.68	35.4±0.56	9.9±0.14
Yeast + 50 mg % papain + boiled milk	4	72.4±0.31	17.8±0.23	9.9±0.06
50 mg % papain + DMS	10	45.4±0.67	45.2±0.44	9.3±0.11
50 mg % papain alone	5	46.6±0.63	45.4±0.63	8.0±0.13
Yeast + 50 mg % papain	4	75.6±0.12	16.6±0.10	7.9±0.06
FLOUR C				
Yeast + fresh milk	3	20.7±1.99	40.2±1.72	39.1±1.11
No additions	5	25.9±0.39	35.9±0.73	38.3±0.63
Yeast alone	3	28.6±1.00	37.9±0.66	33.7±1.08
Fresh milk alone	3	36.6±1.49	30.6±0.96	32.7±1.04
50 mg % papain + fresh milk	3	6.8±1.55	70.7±1.76	22.5±1.78
Yeast + 50 mg % papain + fresh milk	4	36.9±1.37	50.6±1.71	12.4±0.53
50 mg % papain alone	8	47.9±0.05	41.6±0.21	10.6±0.49
Yeast + 50 mg % papain	2	85.0	7.7	7.3

cause any breakdown of gluten unless it was in the presence of yeast without milk. Yeast must be classed as a powerful activator of papain, while milk seems to cause inactivation.

In general skim milk had a similar effect on the action of papain whether it was fresh, boiled, or dried. Boiled milk alone had no appreciable effect on the gluten, but fresh milk and dry milk solids both caused some breakdown. The breakdown caused by dry milk solids was definitely inhibited by the addition of 5 mg % of papain. No difference could be found between the effects of good and poor dry milk solids.

Papain in the higher concentration caused much breakdown of gluten, as was to be expected. Here again however the milk, whether it was fresh, boiled, or dried, had an inactivating effect on the papain.

Yeast alone had an activating effect, most of the nitrogen being washed out in the first fraction.

The flour C had a weaker gluten, since the amount of nitrogen left in the insoluble residue was smaller. The series of treatments was not complete but the inactivating effect of fresh skim milk on 50 mg % of papain is evident.

The results of a statistical analysis of the differences between means are presented in Table III. Increases in each fraction due to

TABLE III
COMPARISON OF THE DIFFERENCES BETWEEN MEANS CAUSED BY
ADDITION OF MILK

Basic treatment	Milk added	Differences caused by milk in		
		Wash water	Sodium salicylate	Insoluble residue
		%	%	%
No additions	DMS	+ 4.7†	- 1.8	- 2.8
No additions	Fresh milk	+ 8.3†	- 6.5*	- 1.8
No additions	Boiled milk	+ 5.2†	- 7.2*	+ 2.0
Yeast	DMS	- 0.9†	+ 7.2†	- 7.1†
5 mg % papain	DMS	+ 5.9†	- 9.5†	+ 3.8*
5 mg % papain	Fresh milk	+ 5.8†	- 4.4†	- 1.2
5 mg % papain	Boiled milk	+ 3.6†	- 3.0	- 0.5
50 mg % papain	DMS	- 1.2	- 0.2	+ 1.3†
50 mg % papain	Fresh milk	- 11.0†	+ 7.1†	+ 3.9†
50 mg % papain	Boiled milk	- 7.0†	+ 2.7*	+ 4.1†
5 mg % papain + yeast	DMS	- 0.8	- 10.4†	+ 11.2†
5 mg % papain + yeast	Fresh milk	- 3.2†	- 5.5*	+ 8.7†
5 mg % papain + yeast	Boiled milk	+ 0.2	- 8.8*	+ 8.6*
50 mg % papain + yeast	Fresh milk	- 20.9†	+ 18.8	+ 2.0†
50 mg % papain + yeast	Boiled milk	- 3.2†	+ 1.2*	+ 2.0†

* Values lie beyond 5% level of significance.

† Values lie beyond 1% level of significance.

the addition of milk to the basic treatment listed in the first column are given plus values. In each case when milk was added to flour alone there was a significant increase in the amount of nitrogen washed out in the first fraction, even though all the nitrogen of the milk was assumed to be washed out in this fraction and first subtracted. Apparently this was a peptizing action similar to that of sodium salicylate since there was a decrease in that fraction. No significant change was observed in the insoluble residue fraction. When dry milk solids were added to yeast + flour there was a significant decrease (although small) in the wash-water fraction, a significant increase in the sodium salicylate fraction, and a significant decrease in the insoluble-residue fraction.

When the milks were added to flour + 5 mg % papain there was in each case a significant increase in the wash-water fraction and a

decrease in the sodium salicylate fraction. There was no significant change in the final fraction except when dry milk solids were added, when there was an increase. In each case when the milk was added to 50 mg % papain there was a significant increase in the insoluble residue. The same holds true when milk is added to the combination of yeast and papain. In fact the only significant decrease found in the insoluble residue when milk was superimposed upon a formula was in the case of yeast alone. These facts very definitely show that milk does not activate papain.

The effect of milk on the action of papain in baking is shown in Table IV. The loaf volumes show that milk inhibits the action of papain.

TABLE IV
EFFECT OF MILK AND PAPAIN ON LOAF VOLUME

Baking formula	Loaf volume
	cc
A Control	838 ± 4.5
B 5 mg % papain	576 ± 8.5
C 6% DMS	853 ± 12.4
D DMS + papain	741 ± 2.4
MEAN DIFFERENCES	
A-B	262*
C-D	112*
D-B	165*

* These values lie beyond 1% level of significance.

The results obtained by the second modification of gluten fractionation seem to follow the baking more closely than the results obtained by the first method. The main difference in the results obtained by the two methods was the action of papain + milk on the gluten. In the first modification the breakdown of gluten by milk and papain was equal to the sum of the breakdowns caused by milk and papain alone. There appeared to be a neutralizing effect when the second modification was used. We believe that the second modification gives a better picture of what occurs during baking.

The coagulating effect of papain on milk is well known. Balls and Hoover (1937) have suggested the use of the milk-clotting time as a measure of the activity of papain. The effect of milk on the action of papain as observed by us may be due to this coagulating effect. In a blank test (with no flour) the milk was not visibly coagulated by papain in the concentration used in the gluten fractionation work. However, the presence of flour may alter the coagulative effect of papain on milk.

Summary

The results of two methods for gluten fractionation are presented. Both methods show that milk itself breaks down gluten but does not activate the breakdown by papain. The second method shows that milk has an inhibitory effect on the action of papain, which is confirmed by baking results. Milk prevents the activation of papain by yeast.

Literature Cited

- Ashworth, U. S., Golding, N. S., Farrah, G. H., and Miller, D. D.
1942 Chemical factors affecting the baking quality of dry milk solids. I
Correlation of pH and baking score. *Cereal Chem.* 19: 94-101.
- Balls, A. K., and Hale, W. S.
1936 Proteolytic enzymes of flour. *Cereal Chem.* 13: 54-60.
1936a Further studies on the activity of proteinase in flour. *Cereal Chem.* 13: 656-664.
- and Hoover, S. R.
1937 The milk clotting time of papain. *J. Biol. Chem.* 121: 737-745.
- Dill, D. B., and Alsberg, C. L.
1924 Some critical considerations of the gluten washing problem. *Cereal Chem.* 1: 222-246.
- Harris, R. H.
1937 A study of gluten protein fractionation from sodium salicylate solution. I. Durum wheat gluten fractionation. *Cereal Chem.* 14: 695-707.
1938 A study of gluten protein fractionation from sodium salicylate solution. II. Bread wheat gluten fractionation. *Cereal Chem.* 15: 80-90.
1939 A study of gluten protein fractionation from sodium salicylate solution. III. The effect of proteolytic enzymes. *Cereal Chem.* 16: 78-88.
- and Johnson, John, Jr.
1939 A study of gluten protein fractionation from sodium salicylate solution. IV. Effect of proteolytic enzymes, as affected by class of wheat. *Cereal Chem.* 16: 279-289.
1940 Some physical and chemical changes occurring in hard red spring wheat gluten during dough fermentation. *Cereal Chem.* 17: 739-759.
1941 A study of some differences in gluten properties existing between varieties and types of wheat. *Cereal Chem.* 18: 395-413.
- Hildebrand, F. C.
1940 Comparison of methods for the determination of proteolytic activity. II. Studies on malted wheat flours. *Cereal Chem.* 17: 635-639.
- Jørgensen, Holger
1936 On the existence of powerful but latent proteolytic enzymes in wheat flour. *Cereal Chem.* 13: 346-355.
- Landis, Q.
1941 Report on proteolytic enzymes. *J. Assoc. Official Agr. Chem.* 24: 615-617.
- McCalla, A. G., and Rose, R. C.
1935 Fractionation of gluten dispersed in sodium salicylate solution. *Can. J. Research* 12: 346-356.
- Rose, R. C., and Cook, W. H.
1935 Viscosity of gluten dispersed in alkali, acid and neutral solvents. *Can. J. Research* 12: 63-81.

CORRELATIONS BETWEEN CRUDE FIBER AND ASH OF WHEAT SHORTS

J. S. SCHLESINGER

Arkansas City Flour Mills, Arkansas City, Kansas

(Received for publication June 9, 1942)

A search of available literature failed to disclose any technical information on the relation between the crude fiber and ash content of wheat shorts. In work extending over a six-month interval in our laboratory, 42 samples of shorts were analyzed for crude fiber and ash content at four- to five-day intervals, the results being calculated to the 15% moisture basis.

The A. A. C. C. one-filtration method¹ was used for the crude fiber test. The shorts were ground to pass a 1-mm sieve. This is essential, as was demonstrated by preliminary tests. The ash test consisted of incinerating a 1-g sample 75 minutes at 1100°F, the resulting ash being weighed back directly on the pan of a chain-type semimicro balance. The moisture determination was carried out on a 2-g sample heated in an air oven on an aluminum plate at 140°C for 20 minutes. The resultant dried material was, of course, used for the fat analysis and finally for the crude fiber determination.

Table I gives the ash content (X) and the crude fiber content (Y) of the 42 samples. The mean (\bar{X}) of the ash is 3.7300% and the mean (\bar{Y}) of the crude fiber is 5.6583%. The standard deviations (σ_x and σ_y) are 0.2235% and 0.4553%, respectively. It is to be noted that these deviations are a measure of the variation that may be expected in commercial samples, and not of variation due to the measuring technique. Had the object been to determine the variability of the ash and crude fiber tests as such, a series of analyses would have been run repeatedly on one sample. In this connection, the coefficient of variation (CV) for ash was 5.99% and for crude fiber 8.04%, indicating that the crude fiber was relatively more variable than the ash.

The coefficient of correlation by the method of Treloar² was found to be +.8858, a very high and significant positive correlation. Although this correlation is based upon a relatively small group of analyses, the group is sufficiently large and the correlation is significantly high to justify the conclusion that a definite significant positive correlation exists between crude fiber and ash values of shorts. This correlation suggests the possibility of predicting crude fiber values from the ash results (Table I). For this purpose the formula

¹ Methods of Analysis of Cereals and Cereal Products, 1928.

² Alan E. Treloar, *An Outline of Biometric Analysis*, 1936. The formula for standard deviation is found on page 16; that of coefficient of variation also on 16; correlation coefficient, page 47; rectilinear regression, page 56, and error of estimate, page 57.

TABLE I
PREDICTION OF CRUDE FIBER CONTENT IN SHORTS FROM ASH CONTENT

Ash X	Analyzed crude fiber Y	Predicted crude fiber $1.8X - 1.06$	Ash X	Analyzed crude fiber Y	Predicted crude fiber $1.8X - 1.06$
e_o	e_o	e_o	e_o	e_o	e_o
3.09	4.64	4.50	3.75	5.75	5.69
3.34	4.28	4.95	3.75	5.94	5.69
3.36	5.10	4.99	3.77	5.67	5.73
3.44	5.02	5.13	3.77	5.87	5.73
3.47	5.05	5.18	3.78	5.53	5.74
3.48	5.68	5.20	3.78	5.93	5.74
3.52	5.56	5.28	3.84	5.61	5.85
3.53	5.48	5.30	3.84	5.81	5.85
3.54	5.39	5.31	3.86	5.94	5.89
3.55	5.23	5.33	3.87	5.68	5.91
3.59	5.50	5.40	3.88	5.78	5.92
3.65	5.27	5.51	3.89	6.16	5.94
3.65	5.45	5.51	3.90	5.57	5.96
3.66	5.48	5.53	3.90	5.82	5.96
3.66	5.74	5.53	3.92	6.27	6.00
3.67	5.75	5.55	3.98	6.03	6.10
3.68	5.33	5.56	4.01	5.97	6.16
3.69	5.72	5.58	4.04	6.07	6.21
3.70	5.54	5.60	4.06	6.18	6.25
3.70	5.64	5.60	4.18	6.53	6.46
3.70	5.80	5.60	4.22	6.89	6.54
			Mean 3.73	5.6583	5.654

All results are calculated to the 15% moisture basis.

$Y = 1.8X - 1.06$ (where Y equals percent of crude fiber and X equals percent of ash) suggests itself from the data, the constants being determined from the formula of Treloar. From the standard error of estimate we can expect that the average predicted results will not vary more than 0.21% from the actual analyzed values. The formula $Y = 1.8X - 1.06$ should be useful in the routine control of fiber in shorts by enabling the control chemist to determine the relative crude fiber content quickly (in less than 90 minutes) when using the quick ash method previously described.

Preliminary work indicates that a constant can be determined that may be used to predict the probable crude fiber of wheat bran ground to pass a 1-mm sieve.

Conclusion

Evidence is presented which shows that for wheat shorts that are ground to pass a 1-mm sieve the coefficient of correlation between crude fiber and ash is $+0.8858$.

A formula for the prediction of probable crude fiber from the ash values (percent of ash times $1.8 - 1.06$ equals percent of crude fiber) has been derived from the data.

FURTHER OBSERVATIONS ON A CRYSTALLINE WHEAT PROTEIN^{1,2}

A. K. BALLS, W. S. HALE, and T. H. HARRIS

Enzyme Research Laboratory, Bureau of Agricultural Chemistry and Engineering,
U. S. Department of Agriculture, Washington, D. C.

(Read at the Annual Meeting, May 1942)

In recent publications (Stuart and Harris; Balls, Hale, and Harris; and Coulson, Harris, and Axelrod, 1942) the preparation and some of the properties of a new crystalline proteinlike material from wheat flour were described. The crystals are the hydrochloride of a substance that appears to be related to the protamines, being of relatively low molecular weight, rich in arginine and (peculiarly) in cystine. Analyses have shown that it is made up largely, if not entirely, of amino acid residues.

This substance is on the border between true proteins and their degradation products. It is incompletely precipitated by sulfosalicylic acid and by trichloroacetic acid. Measurements of the rate of diffusion indicated a value of about 10,000 for the molecular weight but this method is notoriously prone to error. Analytical data have shown that the molecular weight must be at least 6,000.

The sulfur content of the substance is among the highest known for proteins. This sulfur, present as cystine, may be reversibly oxidized and reduced. In the original plant material at least part exists in the reduced form. Because of the prominence of the sulfur and because of the source of the material it is proposed to name this substance "purothionin" (*πυρος* wheat, *θειον* sulfur).

The present paper presents evidence indicating a peptide nature of linkages between the amino acids that make up most, if not all of the molecule. This evidence is based on the digestibility of the substance with certain proteolytic enzymes—namely with crystalline chymotrypsin, chymopapain, and papain, and with crude papain latex. All of these proteinases or mixtures thereof are known to attack CO—NH linkages, while evidence for their action on bonds of other types appears to be scanty. Exhaustive digestion with these proteinases has been found to cause an extensive breakdown of the crystalline material, as shown by increases in the amino nitrogen determinable by the Van Slyke apparatus. The extent of the degradation varies with the enzyme used and in some cases has accounted for half of the increase in amino nitrogen obtained on hydrolysis with acid. A further argument for the proteinlike nature of the substance may be based

¹ Enzyme Research Contribution No. 78.

² Part of this work was done under Bankhead-Jones, Special Research funds.

on the fact that the smooth-muscle-contracting property disappears on digestion by papain. (Histamine, it may be noted, is not inactivated by similar treatment.) The uterus-contracting property of the new substance from wheat is therefore referable to a configuration scissile by the enzyme, presumably at one or more peptide linkages. The toxic properties of the material depend on the manner and sequence in which certain components nontoxic in themselves are bound together in the molecule.

The technique by which the extent of proteolysis was determined consisted in measuring the increase of free primary amino nitrogen in a Van Slyke apparatus. In the case of the pancreas enzyme, chymotrypsin, this presented no difficulty. The digestion ran very slowly at pH 5.0 in the presence of acetate buffer, and much faster at pH 8.0 with phosphate buffer. Reduction of the substrate accelerated the rate of digestion at pH 5.0. There is evidence that final equilibrium is reached with this enzyme only after considerable time.

Digestion by the papainases is complicated by the presence of large quantities of cystine in the substrate. In the oxidized (S-S) form the substance is not digested at all. Instead the enzyme is markedly inhibited with respect to its milk-clotting power by the presence of the substrate. This inhibition may be removed by the addition of the usual reducing agents employed to activate papain. It seems probable therefore that the substance inactivates papain in much the same manner as would cystine itself. In order to obtain proteolysis with enzymes of the sulfur class it is necessary first to reduce the substrate. When this was done splitting occurred rapidly on the addition of the enzyme. Because this substrate precipitates in even weakly alkaline solutions, reduction with cyanide or sulfite was impractical. Reduction with hydrogen sulfide can be accomplished in acid solution and was therefore employed.

The results of experiments in which digestion was carried on in the presence of dissolved hydrogen sulfide were reported in a recent abstract. These results are incorrect because it was not then realized that the reaction between nitrous acid and hydrogen sulfide in the Van Slyke apparatus would produce enough nitrogen to make an appreciable difference in the extent of digestion as measured. More recent experiments, however, have shown that the amount of nitrogen so generated not only introduces a serious error but also a variable one, for the reason that it is difficult to keep the concentration of hydrogen sulfide constant during the course of a digestion lasting many hours.

Instead of applying corrections derived from blank experiments the following technique was used and gave satisfactory results.

"Purothionin" hydrochloride was dissolved in water and diluted with 0.1M acetate buffer (pH 5.0) to give a concentration near 10 mg per ml. The solution was then saturated with hydrogen sulfide for several hours, usually overnight. The hydrogen sulfide was then removed by a stream of hydrogen. The solution was then cooled in ice and to it was added a solution of the enzyme, made two-thirds molar with respect to potassium cyanide five minutes before being used. A portion of the chilled mixture was then immediately placed in the Van

TABLE I
INCREASE IN AMINO NITROGEN DURING DIGESTION WITH PROTEINASES¹

Enzyme	Substrate ²	Enzyme quantity	Amino nitrogen at hours shown ⁴						Increase in amino N
			0	3	20	25-30	45	80	Total amino N after acid hydrolysis
	mg NH ₂ -N	M.C. units ⁵	mg						%
Chymotrypsin, cryst.									
(substrate in S-S form ⁶)	1.14	1.2	0.21	—	0.33	—	—	—	11
(substrate in S-S form)	2.28	1.2	0.33	0.34	—	—	0.37	—	2
(substrate in red. form)	1.21	1.2 ⁶	0.29	0.32	0.39	0.40	0.42	—	11
(substrate in red. form)	2.36	1.2	0.34	—	0.55	0.59	0.61	—	11
(substrate in red. form)	1.21	1.2	0.20	0.25	0.32	0.34	—	—	12
Papain, cryst.	1.14	0.15	0.28	0.55	0.73	—	0.73	—	40
Chymopapain, cryst.	1.14	1.60	0.30	0.51	0.73	—	0.79	0.81	45
Papaya latex	1.14	0.33	0.34	0.56	0.76	0.76	—	—	37

¹ Analysis of 1 ml of digestion mixture at pH 5.0 containing 0.1M acetate and substrate as shown.

² The substrate used is expressed as mg of total amino nitrogen after acid hydrolysis.

³ A milk-clotting unit is taken as the quantity of enzyme that, in a volume of 1 ml, clots 5 ml of an emulsion of powdered milk in one minute at 30°C. The emulsion is made by stirring 20 g of powdered milk with 85 ml of water containing 10 ml of 2M acetic acid + 1M sodium hydroxide buffer (pH 4.6).

⁴ Time approximate only.

⁵ At pH 8.0 in 0.1M phosphate buffer. Digestion by chymotrypsin was very slow at pH 5.0 with the S-S form of the substrate but quite rapid after reduction.

⁶ The enzyme in this experiment was previously treated with KCN in the manner described for the activation of papain. This run is omitted from Table II.

Slyke apparatus (already prepared for use) for the determination of the initial amino nitrogen. The enzyme-substrate mixture was next placed in a thermostat at 35° and maintained there throughout the digestion. The splitting was measured by the withdrawal at intervals of further samples on which similar determinations of amino nitrogen were made. Subsidiary experiments showed that traces of hydrogen sulfide did not materially disturb the results obtained and that the presence of the cyanide (used for activating the enzyme) increased the rate, but not the extent, of digestion. The amino nitrogen produced

by autolysis in the enzyme preparations themselves was carefully determined in each case under the same experimental conditions. It was found to be too small to affect the results. The necessary corrections (amounting in the largest instance to 0.02 mg nitrogen) have been subtracted in the calculations.

The data of the experiments are shown in Table I. The increase in amino nitrogen caused by chymotrypsin was about one-third of that referable to the papainases. Papain and chymopapain apparently

TABLE II
PEPTIDE NITROGEN¹ SET FREE BY DIGESTION

	Peptide N	Proportion
	%	equivalents
Set free by:		
Acid hydrolysis	100	6.0
Chymotrypsin (1) S-S form of purothionin	12	0.7
(2) SH form	13	0.8
(3) SH form	13	0.8
Papain	46	2.7
Chymopapain	51	3.1
Latex	43	2.6
Content of constituents:		
Total chlorine (6.5% of substance)	—	1.5
Peptide N in arginine (20.4% of substance)	—	0.9
Total sulfur (4.42% of substance)	—	1.1
Original amino N (1.65% of substance)	—	0.9

¹ Calculated as total N—($\frac{3}{4}$ arginine N + original amino N).

split the substrate at the same linkages, because digestion mixtures made with papain and chymopapain, respectively, and already at a standstill, showed no increase after being mixed together. Crude papain digested the substrate to the same extent as the crystalline enzymes prepared from it.

The meaning of these results is not very clear, however, until account has been taken of the fact that the original material contains 1.65% of free amino nitrogen. If the original amino nitrogen plus three-fourths of the arginine nitrogen is subtracted from the total nitrogen, 62.1% of the total remains. This residual value has been assumed to represent the nitrogen present in peptide linkages, and is hereafter referred to as "peptide nitrogen." It amounts to 10.8% of the original substance. This assumption is supported by the observation that the amino nitrogen found after hydrolysis with acid agrees very closely with that obtained by calculation from the arginine content determined by an independent method. Moreover, the original amino nitrogen is very nearly a simple submultiple (about one-sixth) of the peptide nitrogen.

Table II shows the increase in amino nitrogen due to digestion expressed as percentage of the peptide nitrogen of the substrate. The nitrogen equivalents of the chlorine, sulfur, and certain other constituents are also shown, calculated on the same basis. Changes in the peptide chain are thus more clearly seen.

From this table it appears that hydrolysis by chymotrypsin has approximately doubled the amount of amino nitrogen originally present. The newly formed amino nitrogen is moreover equivalent to the substrate's content of sulfur and also of arginine. The effect of the papainases is close to thrice that of chymotrypsin.

A simple numerical relationship appears to exist between these values. It is admittedly risky to rely too heavily on data obtained by enzyme action on a possibly inhibitory substrate, especially when only one method of determination has been used. In this case, however, the data correlate remarkably well with values for other constituents (chlorine, sulfur, etc.) determined by methods admittedly very accurate. It is apparent that the data of Table II are best correlated as submultiples of six. Other small integers give poorer agreement.

With chymotrypsin the protein behaves as though one out of every 6 peptide linkages were split; with the papainases, 3 bonds were split out of 6. Furthermore, on the same basis there appears to be one sulfur atom, one arginine residue, and one free amino group for every 6 bonds; while for every 12 there are 3 chlorine atoms.

Evidence is thus fairly satisfactory that at least half of the nitrogen linkages in the substrate are like those occurring in proteins. It is, of course, not necessary to suppose that degradation by papain has been uniformly to dipeptides. It is, however, improbable that such results would have been obtained if the proteinases attacked bonds irregularly placed with respect to the free amino group, the sulfur or the arginine. An attack at regularly recurring intervals throughout the substrate molecule seems to be clearly indicated.

Literature Cited

- Balls, A. K., Hale, W. S., and Harris, T. H.
1942 A crystalline protein obtained from a lipoprotein of wheat flour. *Cereal Chem.* 19: 279-288.
- Coulson, E. J., Harris, T. H., and Axelrod, B.
1942 Effect on small laboratory animals of the injection of the crystalline hydrochloride of a sulfur protein from wheat flour. *Cereal Chem.* 19: 301-307.
- Stuart, L. S., and Harris, T. H.
1942 Bactericidal and fungicidal properties of a crystalline protein isolated from unbleached wheat flour. *Cereal Chem.* 19: 288-300.

BOOK REVIEW

Volumetric Analysis, Volume I: I. M. Kolthoff and V. A. Stenger. Interscience Publishers, Inc., New York. Price \$4.50.

This book was originally published in German in 1927 and an English translation was made by Prof. N. H. Furman and published in 1928. In 1930 a somewhat revised German edition appeared.

The present edition was prepared in English with some change in emphasis and with deletions and additions. It follows the general plan of its predecessors. Its scope is shown by the chapter titles: Fundamentals of Volumetric Analysis, The Principles of Neutralization and Ion Combination Reactions, Titration Curves for Neutralization and Ion Combination Reactions, The Principles of Oxidation-Reduction Reactions, Titration Curves, Indicators, Titration Error, Reaction Velocity, Catalysis and Induced Reactions, Adsorption and Coprecipitation Phenomena, Volumetric Methods of Organic Analysis, Methods for the Determination of the Equivalence-Point.

The chapter on indicators has been expanded and greater emphasis has been placed on oxidation-reduction indicators. Two sections of this chapter deal with acid-base and precipitation indicators. The authors consider indicators largely from the theoretical standpoint and present a good treatise for one interested in the development and use of volumetric analysis. A selected list of indicators having oxidation potentials between 0.24 and 1.30 (against the normal hydrogen electrode) is included, and also a table giving the transition intervals at 18° and 100°C for 16 acid-base indicators. Fifty-five pages are devoted to this chapter.

The following chapter contains a discussion of titration error, primarily the error that occurs because an indicator changes a little before or after the equivalence-point. Equations for the estimation of this error are developed.

A section on the formation of mixed crystals gives a detailed quantitative discussion of the errors involved. Mixed crystal formation is suggested as a method for precipitation of traces of ions from solution for subsequent volumetric determination.

A chapter of 49 pages presents methods of organic analysis. Only the non-electrolytes are discussed. Methods of saponification or hydrolysis of esters, the formation of addition and condensation products, substitution reactions and methods of oxidation and reduction are treated. The chapter suggests that further fundamental studies are needed in this field. No attempt has been made to include special procedures.

In the chapter on the determination of the equivalence-point, the authors have introduced a discussion of the principles of amperometric (polarimetric) titration with polarized electrodes.

The appendix includes among other things a table of ionization constants and solubility products.

The comprehensive treatise of the theoretical aspects of volumetric analysis will interest chemists engaged in developing new methods of analysis or adapting old methods to meet new demands. The theoretical discussions are designed to aid the analyst in the critical examination of proposed reactions to determine their suitability for quantitative titrations. However, the authors emphasize the fact that only by careful investigation can theoretical deduction be put to test.

This book will be a worthy addition to the library of the analytical chemist, and most certainly should be of use as a text or reference book for courses in advanced analytical chemistry.

K. T. WILLIAMS, Western Regional Research Laboratory,
Bureau of Agricultural Chemistry and Engineering,
U. S. Department of Agriculture,
Albany, California

INDEX TO VOLUME XIX

	Page
Action of	
An oxidizing agent in bread dough made from patent flours. Baker, Parker, and Mize.	334
Hydrogen peroxide on wheat flour when used in combination with reducing agents. Elion	594
"Alkali number" of starch as criterion of degree of cooking. Benham	597, 599
"Alkali numbers" of various cereal products. Benham	600
Alpha-amylase in malt evaluation. Kneen and Sandstedt	190
Alsberg, Carl Lucas, memorial to. M. K. Bennett	721
Amylase	
And protease systems of malted wheat flour. Hildebrand and Burkert.	27
In germinating wheat, influence of temperature on. Kneen, Miller, and Sandstedt.	11
Amylopectin in starch. Kerr, Trubell, and Severson	66
Amylose in starch. Kerr, Trubell, and Severson	70
Andrews, A. C. (See C. O. Swanson)	102
Andrews, John S., Harold M. Boyd, and David E. Terry. Riboflavin content of cereal grains and bread and its distribution in products of wheat milling.	55
Anker, C. A., and W. F. Geddes, with C. H. Bailey. Study of the net weight changes and moisture content of wheat flour at various relative humidities	128
Anker, C. A.	
(See W. F. Geddes)	605
(See F. C. Hildebrand)	805
Apparatus	
Fermentometer for gas production. Glabe	230
Flour particle size measurement. Hildebrand, Ferrari, Borchardt, and Anker	811, 812
Gluten testing device. Baker, Parker, and Mize	338
Application of various baking test methods to the evaluation of soft wheat. Shellenberger, Hodler, and Nelson	367
Ash in shorts as correlated with crude fiber. Schlesinger	838
Ashworth, U. S., N. S. Golding, G. H. Farrah, and D. D. Miller. Chemical factors affecting the baking quality of dry milk solids. I. Correlation of pH and baking score	94
Ashworth, U. S., and Herbert A. Harland. Chemical factors affecting the baking quality of dry milk solids. II. Effect of milk on gluten fractionation	830
Atkin, Lawrence. (See Alfred S. Schultz)	529, 532
Axelrod, B. (See E. J. Coulson)	301
Ayre and Anderson proteolytic activity method, study of. Hildebrand	263
Bactericidal and fungicidal properties of a crystalline protein isolated from unbleached wheat flour. Stuart and Harris.	288
Bailey, C. H.	
(See C. A. Anker)	128
(See Olof E. Stamberg)	507
Baker, H. W., and N. L. Gregory. Some preliminary studies pertaining to the operation and use of the Buhler mill.	267
Baker, J. C., and M. D. Mize. Relation of fats to texture, crumb and volume of bread	84
Baker, J. C., H. K. Parker, and M. D. Mize. Action of an oxidizing agent in bread dough made from patent flours	334
Baking	
Effect of, on thiamin. Schultz, Atkin, and Frey	534-537
Ingredients and manipulations, effects of, on Farinograph curves. Moore and Herman	568
Quality, effect of heat treatment of milk on. Stamberg and Bailey	507
Quality of milk solids. Ashworth, Golding, Farrah, and Miller	94
Qualities of spring wheats, as determined by the gluten-starch blend baking methods. Harris and Sibbitt	763
Test for soft wheat. Shellenberger, Hodler, and Nelson	367
Tests and the evaluation of new wheats. Bayfield and West	493

	Page
Balls, A. K., W. S. Hale, and T. H. Harris	
Crystalline protein obtained from a lipoprotein of wheat flour . . .	279
Further observations on a crystalline wheat protein . . .	840
Barackman, R. A. Thiamin retention in self-rising flour . . .	121
Barley kernel changes during malting. Dickson and Burkhart . . .	251
Barlow, O. W. (See George J. Laemmle) . . .	540
Bayfield, E. G., and Glenn A. West. Baking tests and the evaluation of new wheats . . .	493
Bayfield, E. G. (See Glenn A. West) . . .	481
Benham, G. H. Method of determining degree of cooking in cereal products . . .	597
Bennett, M. K. Memorial to Carl Lucas Alsberg . . .	721
Berliner-Schmidt method for sugars in flour. Ziegler . . .	587
Bina, Albert F.	
(See Elmer B. Brown) . . .	447
(See James M. Thomas) . . .	173
Binnington, D. S. Collaborative study of various methods for estimating the pigment content of flour extracts . . .	169
Biochemical properties of "salting-rising" doughs. Milner and Geddes . . .	723
Biscuit and cracker . . .	
Flour testing, committee report on. Loving . . .	358
Flours, statistical study of data on. Tarnutzer . . .	518
Biscuits, thiamin retention in. Barackman . . .	121
Book reviews . . .	
Advances in colloid science, Vol. 1, by Kraemer, with Bartell and Kistler. Reviewed by Harold P. Lundgren . . .	632
Chemical formulary, Vol. V, by Bennett. Reviewed by R. T. Prescott . . .	314
Modern bread from the viewpoint of nutrition, by Sherman and Pearson. Reviewed by H. S. Olcott . . .	538
Volumetric analysis, Vol. 1, by Kolthoff and Stenger. Reviewed by K. T. Williams . . .	845
Borchardt, L. F. (See F. C. Hildebrand) . . .	805
Boyd, Harold M. (See John S. Andrews) . . .	55
Bread . . .	
Manufacture, thiamin stability in. Schultz, Atkin, and Frey . . .	532
Microscopic study of. Burhans and Clapp . . .	196
Properties, relation of fats to. Baker and Mize . . .	84
Riboflavin content of. Andrews, Boyd, and Terry . . .	55, 60, 62
Sodium chloride in. Irvin . . .	346
Brimhall, B., and R. M. Hixon. Interpretation of viscosity measurements on starch pastes . . .	425
Bromate . . .	
As factor influencing wheat germ in baking. Snuth and Geddes . . .	785
Effect, milk solids and. West and Bayfield . . .	481
Bromide residues in cereals fumigated with methyl bromide. Roehm, Shrader, and Stenger . . .	235
Brown, Elmer B. (See James M. Thomas) . . .	173
Brown, E. B., and B. H. Nissen. Effect of temperature and age on malt syrup . . .	243
Brown, Elmer B., James M. Thomas, and Albert F. Bina. Nature of the interfering chromogens encountered in the determination of nicotinic acid in cereal products . . .	447
Brown, R. O. (See J. T. Flohil) . . .	81
Buhler mill, operation of. Baker and Gregory . . .	267
Burhans, Merton E., and John Clapp. Microscopic study of bread and dough . . .	196
Burkhart, B. A. (See Allan D. Dickson) . . .	251
Burkert, G. M. (See F. C. Hildebrand) . . .	27
Cake flour . . .	
Quality, granulation as factor in. Hanson . . .	353
Testing, committee report on. Montzheimer . . .	351
Calculated blank for the estimation of thiamin by the fermentation method. I. Enriched bread. Laemmle and Barlow . . .	540
Canadian wheat, variability of thiamin content of. Johansson and Rich . . .	308
Carlson, Raymond E. (See Henry C. Reitz) . . .	423

	Page
✓ Cereal grains and products, nicotinic acid in. Thomas, Bina, and Brown.	173
Changes in the barley kernel during malting: chemical comparisons of germ and distal portions. Dickson and Burkhart	251
Chemical factors affecting the baking quality of dry milk solids.	
I. Correlation of pH and baking score. Ashworth, Golding, Farrah, and Miller.	94
II. Effect of milk on gluten fractionation. Ashworth and Harland	830
Clapp, John. (See Merton E. Burhans)	196
Collaborative study of	
The applicability of microbiological and chemical methods to the determination of niacin (nicotinic acid) in cereal products Melnick	553
The Ayre and Anderson method for the determination of proteolytic activity. Hildebrand	263
Various methods for estimating the pigment content of flour extracts. Binnington	169
Comparative baking qualities of hard red spring wheat starches and glutens as prepared by the gluten-starch blend baking method. Harris and Sibbitt	763
Cooking cereal products, criterion of degree of. Benham	597
Corn	
Growth-promoting value of, as affected by storage. Jones, Divine, and Gersdorff	819
Products, moisture determination on, critical studies of. Sair and Fetzer	633-720
Proteins of, as affected by storage. Jones, Divine, and Gersdorff	819
Proteins, properties and nutritive value of, as affected by storage. Jones, Divine, and Gersdorff	819
Storage, effect of, on proteins. Jones, Divine, and Gersdorff	819
Vitamin A carotenoids in. Kemmerer, Fraps, and Mangelsdorf	525
Correlations between crude fiber and ash of wheat shorts. Schlesinger	838
Coulson, E. J., T. H. Harris, and B. Axelrod. Effect on small laboratory animals of the injection of the crystalline hydrochloride of a sulfur protein from wheat flour	301
Crude fiber in shorts as correlated with ash. Schlesinger	838
Crystalline flour protein	
Bactericidal and fungicidal property of. Stuart and Harris.	288
Effect of injections of, on small animals. Coulson, Harris, and Axelrod	301
Obtained from a lipoprotein of wheat flour. Balls, Hale, and Harris.	279
Determination of	
Flour particle size. Hildebrand, Ferrari, Borchardt, and Anker	805
Moisture in the wet milling industry. Sair and Fetzer	633-720
Dextrin fermentability. Glabe	442
Dextrins: the relation of fermentability to solubility and reducing power. Glabe	442
Dickson, Allan D. Report of the malt analysis standardization committee	249
Dickson, Allan D., and B. A. Burkhart. Changes in the barley kernel during malting: chemical comparisons of germ and distal portions	251
Dickson, Allan D., and H. L. Shands. Influence of the drying procedure on malt composition	411
Dickson, Allan D. (See H. L. Shands)	471
Dickson, James G. (See H. L. Shands)	471
Distribution of	
Phytic acid in wheat and a preliminary study of some of the calcium salts of this acid. Hay	326
Thiamin in the wheat plant at successive stages of kernel development. Geddes and Levine.	547
Divine, J. P. (See D. Breese Jones)	819
Dixon, H. B. (See Lawrence Zeleny)	1
Dough	
Microscopic structure of. Burhans and Clapp	196
Oxidizing action on. Baker, Parker, and Mize	334
Physical properties of. Swanson and Andrews	102
Reworking of. Frank	379
Softening effect of malted wheat flour on. Hildebrand and Burkert	31
Doughs, protease activity and reducing matter of. Shen and Geddes	609

	Page
Durum	
Milling equipment. Harris and Sibbitt	388
Processing equipment, experimental. Harris and Sibbitt	390-392
Quality as affected by blight. Harris and Sibbitt	403
Quality, effect of wheat damage on. Harris and Sibbitt	403
Wheats, North Dakota, quality of. Harris and Sibbitt	388, 403
Effect of	
Certain ingredients and variations in manipulations on the Farinograph curve. Moore and Herman	568
Heat treatment of milk in relation to baking quality as shown by Polarograph and Farinograph studies. Stamberg and Bailey	507
Storage of corn on the chemical properties of its proteins and on its growth-promoting value. Jones, Divine, and Gersdorff	819
Temperature and age on malt syrup. Brown and Nissen	243
Temperature change during malting on four barley varieties. Shands, Dickson, and Dickson	471
Temperature upon the viability and baking properties of dry and moist yeast stored for varied periods. Thiessen	773
Effect on small laboratory animals of the injection of the crystalline hydrochloride of a sulfur protein from wheat flour. Coulson, Harris, and Axelrod	301
Effectiveness of dry milk solids in preventing overbromation of some bleached hard winter wheat flours. West and Bayfield	481
Elion, E. Action of hydrogen peroxide on wheat flour when used in combination with reducing agents	594
Enzymes in malted wheat flour. Hildebrand and Burkert	27
Evaluation of	
Fluorophotometers to be used in the thiochrome assay for vitamin B ₁ . Loofbourow and Harris	151
Malt for use as a flour supplement. Kneen and Sandstedt	181
Experimental	
Baking of salt rising bread. Milner and Geddes	726
Durum milling and processing equipment with further quality studies on North Dakota durum wheats. Harris and Sibbitt	388
Milling data, interpretation of. Rumold	384
Milling with Buhler mill. Baker and Gregory	267
Factors which influence the physical properties of dough. IV. Effects of surface active agents on the characteristics of the curves made by the recording dough mixer. Swanson and Andrews	102
Farinograph	
Curve, effects of baking ingredients and manipulations on. Moore and Herman	568
Studies of heat treatment of milk in relation to baking quality. Stamberg and Bailey	507
Farrah, G. H. (See U. S. Ashworth)	94
Fats, relation of, to bread properties. Baker and Mize	84
Fermentation method for thiamin, blank for. Laemmle and Barlow	540
Fermentometer	
A gas-production measuring device. Glabe	230
For measuring gas production. Glabe	230
Ferrari, C. G. (See F. C. Hildebrand)	805
Fetzer, W. R. (See L. Sair)	633
Flohil, J. T., and R. O. Brown. Quantitative control test for iron added to flour	81
Flour	
Iron in. Flohil and Brown	81
Lipoprotein, crystalline protein from. Balls, Hale, and Harris	279
Moisture at various humidities. Anker, Geddes, and Bailey	128
Moisture determinations, precision of. Geddes and Anker	605
Particle size, determination of. Hildebrand, Ferrari, Borchardt, and Anker	805
Weight changes at various humidities. Anker, Geddes, and Bailey	128
Fluorophotometers, evaluation of. Loofbourow and Harris	151

	Page
Frank, E. N. Some effects of reworking doughs	379
Fraps, G. S. (See A. R. Kemmerer)	525
Frey, Charles N. (See Alfred S. Schultz)	529, 532
Further developments in the photometric determination of wheat protein.	
Zeleny, Neustadt, and Dixon	1
Further observations on a crystalline wheat protein. Balls, Hale, and Harris	840
Gas production, measuring device for. Glabe	230
Geddes, W. F., and C. A. Anker. Precision of flour moisture results between and within laboratories	605
Geddes, W. F.	
(See C. A. Anker)	128
(See Max Milner)	723
(See Ting Shen)	609
(See Donald E. Smith)	785
Geddes, W. F., and M. N. Levine. Distribution of thiamin in the wheat plant at successive stages in kernel development	547
Germinating wheat, amylase in. Kneen, Miller, and Sandstedt	11
Gersdorff, Chas. E. F. (See D. Breese Jones)	819
Glabe, Elmer F.	
Dextrins: relation of fermentability to solubility and reducing power	442
Fermentometer, a gas-production measuring device	230
Gluten fractionation, effect of milk on. Ashworth and Harland	830
"Gluten-starch blend" baking method applied to spring wheats. Harris and Sibbitt	763
Golding, N. S. (See U. S. Ashworth)	94
Gortner, Ross Aiken. (See Henry C. Reitz)	423
Granulation as a factor in cake flour quality. Hanson	353
Gregory, N. L. (See H. W. Baker)	267
Hale, W. S. (See A. K. Balls)	279, 840
Hanson, W. H. Granulation as a factor in cake flour quality	353
Harland, Herbert A. (See U. S. Ashworth)	830
Harris, R. H., Raymond V. Olson, and John Johnson, Jr. Viscosity changes in sodium salicylate dispersion of hard red spring wheat gluten in relation to variety and environment	748
Harris, R. H., and L. D. Sibbitt	
Comparative baking qualities of hard red spring wheat starches and glutes as prepared by the gluten-starch blend baking method	763
Experimental durum milling and processing equipment, with further quality studies on North Dakota durum wheats	388
Quality of North Dakota durum wheat as affected by blight and other forms of damage in 1940	403
Utility of micro methods of test-weight determination with hard red spring wheat	458
Harris, Robert S. (See John R. Loofbourov)	151
Harris, T. H.	
(See A. K. Balls)	279, 840
(See E. J. Coulson)	301
(See L. S. Stuart)	288
Hay, J. G. Distribution of phytic acid in wheat and a preliminary study of some of the calcium salts of this acid	326
Herman, Ralph S. (See Claude L. Moore)	568
Hildebrand, F. C. Collaborative study of the Ayre and Anderson method for the determination of proteolytic activity	263
Hildebrand, F. C., and G. M. Burkert. Amylase and protease systems of malted wheat flour	27
Hildebrand, F. C., C. G. Ferrari, L. F. Borchardt, and C. A. Anker. Determination of flour particle size	805
Hixon, R. M. (See B. Brimhall)	425
Hodler, Paul W. (See J. A. Shellenberger)	367
Humidity effects on flour moisture content. Anker, Geddes, and Bailey	128
Hydrogen peroxide action on wheat flour. Elion	594
Hysteresis of air dry wheat starch. Reitz, Gortner, and Carlson	423

	Page
Improved method for the volumetric determination of sodium chloride in bread. Irvin	346
Influence of Temperature on the development of amylase in germinating wheat. Kneen, Miller, and Sandstedt.	11
The drying procedure on malt composition. Dickson and Shands.	411
Interpretation of viscosity measurements on starch pastes. Brimhall and Hixon	425
Interpreting experimental milling data from a commercial aspect. Rumold .	384
Iron in flour, test for. Flohil and Brown	81
Irvin, Roy. Improved method for the volumetric determination of sodium chloride in bread.	346
Johannson, H., and C. E. Rich. Variability in the thiamin content of western Canadian hard red spring wheat of the 1940 crop.	308
Johnson, John A., Jr., and C. O. Swanson. Testing of wheat quality by record- ing dough mixer curves obtained from sifted wheat meals.	216
Johnson, John A., Jr. (See R. H. Harris)	748
Jones, D. Breese, J. P. Divine, and Chas. E. F. Gersdorff. Effect of storage of corn on the chemical properties of its proteins and on its growth-promoting value	819
Kemmerer, A. R., G. S. Fraps, and P. C. Mangelsdorf. Relation between the vitamin-A-active carotenoids in corn and the number of genes for yellow color	525
Kerr, Ralph W., O. R. Trubell, and G. M. Severson. On the multiple amylose concept of starch. II. Amylopectin and amylose.	64
Kneen, Eric, Byron S. Miller, and R. M. Sandstedt. Influence of temperature on the development of amylase in germinating wheat.	11
Kneen, Eric, and R. M. Sandstedt. Evaluation of malt for use as a flour supple- ment.	181
Koch, John R., and Sister Mary Lauretta, S.S.N.D. Viscometric determination of the optimum pH for the proteolytic activity of malt with gelatin as a substrate.	315
Laemmle, George J., and O. W. Barlow. Calculated blank for the estimation of thiamin by the fermentation method. I. Enriched bread.	540
Landis, Quick. Simple method for the approximate estimation of proteolytic activity.	419
Levine, M. N. (See W. F. Geddes)	547
Lipoprotein, crystalline protein from. Balls, Hale, and Harris	279
Loofbourov, John R., and Robert S. Harris. Evaluation of fluorophotometers to be used in the thiochrome assay for vitamin B ₁	151
Loving, H. J. Report of the 1940-41 committee on testing biscuit and cracker flours.	358
Lundgren, Harold P. Book review. Advances in colloid science, Vol. 1, by Kaemer, Bartell, and Kistler.	632
Malt Analysis standardization committee report. Dickson	249
Composition, effect of drying procedure on. Dickson and Shands.	411
Drying, temperature effects in. Dickson and Shands	413, 414
Evaluation of as flour supplement. Kneen and Sandstedt.	181
Proteolytic activity of, optimum pH for. Koch and Lauretta	315
Syrup, effect of temperature and age on. Brown and Nissen	243
Malted wheat flour, amylase and protease in. Hildebrand and Burkert.	27
Malting Barley kernel changes in. Dickson and Burkhardt.	251
Temperature changes during, effect of. Shands, Dickson, and Dickson.	471
Malts, barley, amylases of. Shands, Dickson, and Dickson.	472, 473
Mangelsdorf, P. C. (See A. R. Kemmerer)	525
Melnick, Daniel. Collaborative study of the applicability of microbiological and chemical methods to the determination of niacin (nicotinic acid) in cereal products.	553
Memorial to Carl Lucas Alsberg. Bennett	721
Method of determining degree of cooking in cereal products. Benham	597

	Page
Methods	
Amylase activity. Kneen and Sandstedt	183
Degree of cooking ("alkali number"). Benham	597
Fermentometer for gas production. Glabe	230
Flour particle size. Hildebrand, Ferrari, Borchardt, and Anker	805
Iron in flour. Flohil and Brown	82
Moisture in products of the wet milling industry. Sair and Fetzer	714-720
Pigment content of flour extracts. Binnington	169
Proteolytic activity, simple procedure for. Landis	419
Salt in bread, volumetric method for. Irvin	346
Test-weight, micro method for	
Harris and Sibbitt	458
Swanson	468
Viscometric determination of proteolytic activity. Koch and Laurretta	315
Wheat protein photometric method. Zeleny, Neustadt, and Dixon	2
Yeast, microscopic examination of. Thiessen	783
Methyl bromide fumigation in cereals. Roehm, Shrader, and Stenger	235
Micro method for determining test weight. Swanson	468
Micro method for determination of test weight	
Harris and Sibbitt	458
Swanson	468
Microbiological and chemical methods for nicotinic acid. Melnick	553
Microscopic	
Structure of bread and dough. Burhans and Clapp	196
Study of bread and dough. Burhans and Clapp	196
Milk	
Effect of, on gluten fractionation. Ashworth and Harland	830
Heat treatment of, in relation to baking quality. Stamberg and Bailey	507
Solids, baking quality factors of. Ashworth, Golding, Farrah, and Miller	94
Solids, effect of, in preventing overbromation of flour. West and Bayfield	481
Solids, factors affecting baking quality of. Ashworth and Harland	830
Miller, Byron S. (See Eric Kneen)	11
Miller, D. D. (See U. S. Ashworth)	94
Milner, Max, and W. F. Geddes. Biochemical properties of "salt-rising" doughs	723
Mixing curves	
Effects of surface active agents on. Swanson and Andrews	102
From sifted wheat meals. Johnson and Swanson	216
Mize, M. D. (See J. C. Baker)	84, 334
Modeer, Elmer. Report of the 1940-41 committee on testing self-rising and phosphated flours	364
Moisture determination on	
Corn by-product feeds, critical comparison of methods for. Sair and Fetzer	669-681
Corn gluten. Sair and Fetzer	678
Corn gluten feed, critical comparison of methods for. Sair and Fetzer	692-706
Corn gluten meal, critical comparison of methods for. Sair and Fetzer	681-692
Corn oil meal, critical comparison of methods for. Sair and Fetzer	706-713
Ground corn, critical comparison of methods for. Sair and Fetzer	633-668
Products of the wet milling industry, critical studies of. Sair and Fetzer	633-720
Moisture of flour at different humidities. Anker, Geddes, and Bailey	128
Montzheimer, J. W. Report of the 1940-41 committee on methods of testing cake flour	351
Moore, Claude L., and Ralph S. Herman. Effect of certain ingredients and variations in manipulations on the Farinograph curve	568
Multiple amylose concept of starch. II. Amylopectin and amylose. Kerr, Trubell, and Severson	64
Nature of the interfering chromogens encountered in the determination of	
nicotinic acid in cereal products. Brown, Thomas, and Bina	447
Helson, C. A. (See J. A. Shellenberger)	367
Hylson, J. Wesley, and Leroy Palmer. Thiamin, riboflavin, nicotinic acid and Hysnanthothenic acid contents of wild rice (<i>Zizania aquatica</i>)	539

	Page
Neustadt, M. H. (See Lawrence Zeleny)	1
Niacin. (See nicotinic acid).	
Nicotinic acid	
Content of cereal products. Thomas, Bina, and Brown.	173
Determination, interfering chromogens in. Brown, Thomas, and Bina	447
In cereal products. Brown, Thomas, and Bina.	447
In cereal products. Thomas, Bina, and Brown.	173
Microbiological and chemical methods for. Melnick	553
Nissen, B. H. (See E. B. Brown)	243
Nutritive value of the protein and mineral constituents of	
Dry and wet cultivated rices. Sreenivasan and Sadasivan.	47
Rice varieties. Sreenivasan	36
Olcott, H. S. Book review. Modern bread from the viewpoint of nutrition, by Sherman and Pearson.	538
Olson, Raymond V. (See R. H. Harris)	748
Oxidizing agent, action of, in dough. Baker, Parker, and Mize	334
Palmer, Leroy S. (See J. Wesley Nelson)	539
Parker, H. K. (See J. C. Baker)	334
Phosphoric, phytic acid, in wheat products. Hay	326
Photometric determination of wheat protein. Zeleny, Neustadt, and Dixon	1
Phytic acid in wheat products. Hay	326
Pigment, estimation of, in flour extracts. Binnington	169
Polarograph studies of heat treatment of milk in relation to baking quality. Stamberg and Bailey	507
Precision of flour moisture results between and within laboratories. Geddes and Anker	605
Prescott, R. T. Book review. Chemical formulary, Vol. V, by Bennett.	314
Protease	
Activity and reducing matter content of wheat flour doughs in relation to baking behavior. Shen and Geddes	609
In malted wheat flour. Hildebrand and Burkert.	27
Protein	
Crystalline, from wheat flour. Balls, Hale, and Harris	840
Crystalline, from wheat flour lipoprotein. Balls, Hale, and Harris	279
Soluble, washed from gluten. Baker, Parker, and Mize	341, 342
Proteins, corn, as affected by storage. Jones, Divine, and Gersdorff	822-824
Proteolytic activity	
Method, collaborative study of. Hildebrand	263
Of malt, viscometric determination of. Koch and Lauretta.	315
Simple method for. Landis.	419
"Purothionin," a crystalline protein from flour. Balls, Hale, and Harris	840
Quality of North Dakota durum wheat as affected by blight and other forms of damage in 1940. Harris and Sibbitt	403
Quantitative control test for iron added to flour. Flohil and Brown	81
Reducing matter in flour doughs. Shen and Geddes.	609
Reitz, Henry C., Ross Aiken Gortner, and Raymond E. Carlson. Hysteresis of air dry wheat starch	423
Relation between the vitamin-A-active carotenoids in corn and the number of genes for yellow color. Kemmerer, Fraps, and Mangelsdorf	525
Relation of fats to texture, crumb, and volume of bread. Baker and Mize.	84
Report of	
Malt analysis standardization committee. Dickson	249
1940-41 committee on testing biscuit and cracker flours. Loving	358
1940-41 committee on methods of testing cake flour. Montzheimer.	351
1940-41 committee on testing of self-rising and phosphated flours. Modeer	364
Reworking of fermenting doughs. Frank.	379
Riboflavin	
Content of cereal grains, mill products and bread. Andrews, Boyd, and Terry	55

	Page
✓ Content of cereal grains and bread and its distribution in products of wheat milling. Andrews, Boyd, and Terry	55
Microbiological method for. Andrews, Boyd, and Terry	56
Rice	
Composition of. Sreenivasan.	37, 49
Nutritive value of dry and wet cultivated. Sreenivasan and Sadasivan	47
Varieties, nutritive value of, protein and mineral constituents of. Sreenivasan.	36
Rich, C. E. (See H. Johannson)	308
Roehm, L. S., S. A. Shrader, and V. A. Stenger. Bromide residues in cereals fumigated with methyl bromide.	235
Rumold, Perie. Interpreting experimental milling from a commercial aspect	384
Sadasivan, V. (See A. Sreenivasan)	47
Sair, L., and W. R. Fetzer. Determination of moisture in the wet milling industry.	633-720
"Salt-rising" doughs	
Biochemical properties of. Milner and Geddes.	723
Gas production in. Milner and Geddes.	734-738
Physical properties of. Milner and Geddes.	741-744
Sandstedt, R. M. (See Eric Kneen)	11, 181
Schlesinger, J. S. Correlations between crude fiber and ash of wheat shorts.	838
Schultz, Alfred S., Lawrence Atkin, and Charles N. Frey.	
Stability of vitamin B ₁ in the manufacture of bread.	532
Thiamin content of wheat flour milled by the stone milling process.	529
Self-rising and phosphated flours, committee report on. Modeer	364
Severson, G. M. (See Ralph W. Kerr)	64
Shands, H. L., Allan D. Dickson, and James G. Dickson. Effect of temperature change during malting on four barley varieties.	471
Shands, H. L. (See Allan D. Dickson)	411
Shen, Ting, and W. F. Geddes. Protease activity and reducing matter content of wheat flour doughs in relation to baking behavior.	609
Shellenberger, J. A., Paul W. Hodler, and C. A. Nelson. Application of various baking test methods to the evaluation of soft wheat.	367
Shorts, correlation between crude fiber and ash in. Schlesinger.	838
Shrader, S. A. (See L. S. Roehm)	235
Sibbitt, L. D. (See R. H. Harris)	388, 403, 458, 763
Simple method for the approximate estimation of proteolytic activity. Landis	419
Sister Mary Lauretta, S.S.N.D. (See John R. Koch)	315
Smith, Donald E., and W. F. Geddes. Yeast fermentation and potassium bromate as factors influencing the harmful effects of wheat germ on baking quality.	785
Sodium chloride in bread. Irvin.	346
Sodium salicylate dispersion	
Of spring wheat gluten in relation to variety and environment. Harris, Olson, and Johnson	748
Rates for some spring wheat glutes. Harris, Olson, and Johnson.	751-759
Soft wheat, evaluation of, by baking tests. Shellenberger, Hodler, and Nelson.	367
Some effects of reworking fermenting doughs. Frank.	379
Some preliminary studies pertaining to the operation and use of the Buhler mill. Baker and Gregory	267
Sreenivasan, A., and V. Sadasivan. Nutritive value of the protein and mineral constituents of dry and wet cultivated rice.	47
Sreenivasan, A. Nutritive value of the protein and mineral constituents of rice varieties.	36
Stability of vitamin B ₁ in the manufacture of bread. Schultz, Atkin, and Frey.	532
Stamberg, Olof E., and C. H. Bailey. Effect of heat treatment of milk in relation to baking quality as shown by Polarograph and Farinograph studies.	507
Starch	
"Alkali number" of. Benham.	597-599
Fractions, diastatic analysis of. Kerr, Trubell, and Severson.	75
Multiple amylose concept of. Kerr, Trubell, and Severson.	64

	Page
Pastes, viscosity measurements on. Brimhall and Hixon	425
Wheat, hysteresis of. Reitz, Gortner, and Carlson	423
Statistical study of the data of subcommittees on testing biscuit and cracker flours. Tarnutzer	518
Stenger, V. A. (See L. S. Roehm)	235
Stone milling process in relation to thiamin in wheat flour. Schultz, Atkin, and Frey	529
Stuart, L. S., and T. H. Harris. Bactericidal and fungicidal properties of a crystalline protein isolated from unbleached wheat flour	288
Study of the net weight changes and moisture content of wheat flour at various relative humidities. Anker, Geddes, and Bailey	128
Sugars in flour, Berliner-Schmidt colorimetric method for. Ziegler	587
Surface active agents, effects of, on dough properties. Swanson and Andrews	102
Swanson, C. O., and A. C. Andrews. Factors which influence the physical prop- erties of dough. IV. The effects of surface active agents on the character- istics of the curves made by the recording dough mixer	102
Swanson, C. O. A micro method for determining test weight	468
(See John A. Johnson, Jr.)	216
Tarnutzer, Charles A. Statistical study of the data of subcommittees on testing biscuit and cracker flours	518
Terry, David E. (See John S. Andrews)	55
Test weight determination, micro method for Harris and Sibbitt	458
Swanson	468
Testing of wheat quality by recording dough mixer curves obtained from sifted wheat meals. Johnson and Swanson	216
Tests with the Berliner-Schmidt colorimetric method for the rapid determination of sugars in wheat flour. Ziegler	587
Thiamin By fermentation method, blank for. Laemmle and Barlow	540
Content of wheat flour milled by the stone milling process. Schultz, Atkin, and Frey	529
Distribution in wheat plant. Geddes and Levine	547
In stone-milled flour. Schultz, Atkin, and Frey	529
In wheat plant at successive stages of kernel development. Geddes and Levine	547
Retention in self-rising flour biscuits. Barackman	121
Riboflavin, nicotinic acid, and pantothenic acid contents of wild rice (<i>Zizania aquatica</i>). Nelson and Palmer	539
Stability of in bread production. Schultz, Atkin, and Frey	532
Variability in western Canadian wheat. Johansson and Rich	308
Thiessen, E. F. Effect of temperature upon the viability and baking properties of dry and moist yeast stored for varied periods	773
Thiochrome assay for vitamin B ₁ , fluorophotometers for. Loofbourow and Harris	151
Thomas, James M., Albert F. Bina, and Elmer B. Brown. Nicotinic acid content of cereal products	173
Thomas, James M. (See Elmer B. Brown)	447
Trubell, O. R. (See Ralph W. Kerr)	64
Utility of micro methods of test-weight determination with hard red spring wheat. Harris and Sibbitt	458
Variability in the thiamin content of western Canadian hard red spring wheat of the 1940 crop. Johansson and Rich	308
Viscometric Determination of the optimum pH for the proteolytic activity of malt with gelatin as a substrate. Koch and Laurretta	315
Method for proteolytic activity of malt. Koch and Laurretta	315

	Page
Viscosity	
Changes in sodium salicylate dispersion of hard red spring gluten in relation to variety and environment. Harris, Olson, and Johnson.	748
Of starch pastes, measurement of. Brimhall and Hixon.	425
Vitamin A carotenoids in corn. Kemmerer, Fraps, and Mangelsdorf	525
Vitamin B components in wild rice. Nelson and Palmer	539
Vitamin B ₁ stability in bread manufacture. Schultz, Atkin, and Frey	532
West, Glenn A., and E. G. Bayfield. Effectiveness of dry milk solids in preventing overbromation of some bleached hard winter wheat flours	481
West, Glenn A. (See E. G. Bayfield).	493
Wheat	
Germ in baking, influence of yeast fermentation and bromate on. Smith and Geddes	785
Meal, quality tests on. Johnson and Swanson.	216
Plant, thiamin distribution in. Geddes and Levine	547
Protein, photometric determination of. Zeleny, Neustadt, and Dixon.	1
Quality, testing sifted wheat meals for. Johnson and Swanson.	216
Wheats, evaluation of, by baking tests. Bayfield and West	493
Wild rice, vitamin B components in. Nelson and Palmer	539
Williams, K. T. Book review. Volumetric analysis, Vol. 1, by Kolthoff and Stenger.	845
Yeast, baking properties of, as affected by storage temperature and time. Thiessen	773
Yeast	
Fermentation and potassium bromate as factors influencing the harmful effects of wheat germ on baking quality. Smith and Geddes.	785
Viability of, as affected by storage temperature and time. Thiessen.	773
Zeleny, Lawrence, M. H. Neustadt, and H. B. Dixon. Further developments in the photometric determination of wheat protein	1
Ziegler, Edwin. Tests with the Berliner-Schmidt colorimetric method for the rapid determination of sugars in wheat flour.	587

